MOLYBDENUM CONTENT OF PASTURE SPECIES AND SOME FACTORS THAT AFFECT IT

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INTRODUCTION

Among the trace elements in soils that are of importance to agriculture molybdenum is perhaps the most unique. Only a minute amount of this element is required for the normal physiological processes of plants. Although the amount needed is small, this element has been reported as deficient in some areas. However, applications of as little as one ounce to two pounds molybdenum per acre have been found to be sufficient to supply crop needs on most soils. In general, this element does not present a serious problem as far as plant nutrition is concerned because it is usually well distributed in soils, and it is available in sufficient quantity to meet the normal requirements of plants. In direct contrast to the above molybdenum can also be taken up and accumulated by plants in amounts that are toxic to animals feeding directly on such herbage. This role of molybdenum in causing a wide nutritional imbalance in livestock is well recognized and has received wide interest from researchers in animal nutrition.

That molybdenosis, "teart" or scouring in cattle and sheep is attributed to animal ingestion of herbage or pasture crops high in molybdenum content has been known for over 20 years. Forage crops grown on the teart pastures are much higher in molybdenum than those grown on non-teart pastures. The symptoms of the so-called disease condition which develop in cattle grazing on high molybdenum pastures are similar to those caused

by copper deficiency. That the symptoms caused by excess molybdenum can be eliminated by dosing the affected animals with copper sulfate indicates a direct antagonism between these two elements. Excess molybdenum may inhibit either the absorption or utilization of copper in the animal body, thus limiting the copper supply for normal physiological processes. This situation is greatly aggravated when the forage contains both a low level of copper and a high level of molybdenum.

As one might suspect, the molybdenum content of plants varies greatly from one species to another, and even within a single species when grown under different conditions. Seasonal variation is one of the factors responsible for the fluctuation in content of this element in plants. The influence of seasonal change can be explained in terms of changes in the availability of soil molybdenum and by differences in stage of growth and size of the plants at a particular time. In addition to the climatic factors, the content of the element in the plants can be altered greatly by the presence of other nutrients in the soil through the various interactions among ionic species. Molybdenum is taken up by plants as the molybdate anion. Among the other anions present in the soil solution, sulfate and phosphate are two nutrient anions that are known to influence the mechanism of uptake of molybdenum by plants. Hydroxyl ions, although known to play an important role in influencing the molybdenum nutrition of the plant, do not seem to interfere with the mechanism of uptake of molybdenum, but rather, have a strong influence on the availability of the

element in the soil. One important reason for liming acid soils is to correct deficiency symptoms of molybdenum in plants. Liming increases the hydroxyl ion activity and thus increases the anion exchange activity between OH and MoO_4, resulting in the release of molybdate anions into soil solution. Considering the uptake mechanism of molybdenum as influenced by sulfate and phosphate anions, sulfate decreases the molybdenum uptake of plants by competing strongly for the absorption sites on the plant roots. On the other hand, phosphate enhances the uptake and translocation of this element. The best explanation given so far is that phosphate increases the rate of energy-producing biochemical processes, thereby increasing the capacity of the plant to utilize molybdenum from the soil solution. Thus, the rate and capacity of molybdenum uptake by the plant is enhanced. It is also suggested that phosphate might be involved in the anion exchange process in the soil, thus making the molybdate anion more available.

About a decade ago, Fujimoto and Sherman (1951) made a survey of the content of molybdenum in various plant species grown in the Hawaiian Islands and found no plant species having a molybdenum content greater than 2.5 ppm. This value is well below the level of molybdenum (6 ppm.) considered to be toxic in California (Britton and Goss, 1946).

Recently there have been reports on the possibility of molybdenum toxicity occurring in certain areas of the Hawaiian Islands. These reports point to the need for the present investigation which is concerned with a study

of the quantity of molybdenum in pasture plants, some factors that influence the uptake of molybdenum by plants, and also its availability in the associated soils.

The overall thesis will be divided into four different phases which relate closely with one another.

PHASE I. Seasonal variation in molybdenum content of pasture species. This study will provide information on the level of Mo in pasture species grown on rangelands on the Island of Hawaii as influenced by changes in season, rainfall, elevation, and also soil type.

PHASE II. A preliminary survey of the molybdenum and copper status of some pasture species and associated soils on the Island of Hawaii. The investigation is purely exploratory as to the level of copper and molybdenum in pasture plants and the associated soils. However, this phase attempts to investigate the soil-plant relationships, if any, regarding molybdenum and copper. The possibility of toxic levels of molybdenum and/or copper deficiency will also be indicated.

PHASE III. Molybdenum uptake by plants as affected by molybdate, phosphate, and sulfate fertilization. The objective of this experiment is to determine the effects of phosphate and sulfate on the uptake of molybdenum from soils, and the extent to which molybdenum application increases the molybdenum content of the plant.

PHASE IV. Availability of molybdenum in simplified soil systems as influenced by the pH of the system and by phosphate and sulfate concentrations. This part of the thesis is associated closely to the third phase. An attempt is made to determine the mechanisms involved in the influence of molybdate-phosphate-sulfate inter-relationships in soil systems on the availability of molybdenum in the soil solution.

REVIEW OF LITERATURE

History and Role of Molybdenum in Plant Nutrition

A relation between molybdenum and biological systems was not recognized until 1930, when the first evidence was given by H. Bortels of Germany. He considered molybdenum as a catalyst that aided the conversion of gaseous nitrogen to a usable form by nitrogen fixing bacteria. After extensive determinations of the content of this element in land and aquatic materials, Meulen (1932) postulated that molybdenum was necessary for plant life. Bortels (1937) had also noted that certain legumes treated with molybdenum were darker green than the control plants, and suggested the possible importance of molybdenum in the symbiotic fixation of nitrogen by Leguminoseae. Jensen and Betty (1943) discovered that molybdenum increased the percentage nitrogen in the roots of lucerne, as well as the yield of tops. Bortels (1940), again, found that molybdenum increased nitrogen fixation by pure cultures of Anabaena and Nostoc and were also required when Anabaena azollae was grown in symbiosis with Azolla carrolinina. Steinberg (1937) suggested that molybdenum may be required in nitrate reduction, for when nitrate nitrogen was supplied to Aspergillus niger, molybdenum was required to a greater degree than when ammonia or organic nitrogen was used. Arnon and Stout (1939) were able to show that tomato plants could not complete their life cycle when grown in a medium containing all other essential

elements except molybdenum, and that the plants could complete their life cycle when sodium molybdate was added to the solution in an amount equivalent to 0.01 ppm. molybdenum. In 1940 two similar experiments were conducted by Hoagland (1940), using plum tree seedlings, and by C. S. Piper (1940). That molybdenum is involved directly in the metabolism of higher plants was confirmed by the response of non-legumes to molybdenum in water culture from which traces of this element had been adequately eliminated. These included tomatoes (Arnon and Stout, 1949), lettuce (Arnon, 1940), oats (Piper, 1940), lettuce (Brenchley and Warington, 1942), white mustard (Arnon, 1940), and myrobalan plum seedling (Hoagland, 1941).

In plant metabolism, one role of molybdenum is either directly or indirectly associated with nitrate reduction. Spencer and Wood (1954) found that when a molybdenum deficient plant was treated with molybdenum, the amount of nitrate and ammonia was doubled four hours after treatment and the accumulation of nitrate decreased considerably. In the same year, Nicholas, et al (1954a, b) was able to provide the first evidence that molybdenum was associated with nitrate reductase enzymes. Without molybdenum in the highly purified media, Neurospora crassca and Aspergillus niger showed a striking reduction in nitrate reductase activities, and the activities were restored to the normal level 12 hours after treated with molybdenum while other metals added did not evidence a similar capacity.

Availability of Molybdenum in the Soil

Molybdenum is taken up by plant roots through the process of anion adsorption as in the case of sulfate and phosphate. The most available form in the soil is the divalent molybdate anion. The trioxide form, while not available as such, is readily rendered so by reactions with cations in the soils (Styles, 1961).

F. B. Davies (1956), in his discussion, divided the forms of molybdenum in soils into four categories. The first one is "unavailable molybdenum" which is held within the crystal lattice of primary and secondary minerals. The second is "conditionally available molybdenum" which is retained as the MoO_4 anion by clay minerals and "available" to a greater or lesser degree depending on pH and "possibly" on the phosphate status of the soil. The third form is in organic matter, and the fourth is water-soluble molybdenum which assumes a very small magnitude in a given soil. Stephen and Oertel (1943) early suggested that the effect of increasing pH on increasing the availability of molybdenum could be due to the increase in OH ions and thus exchanging with MoO_4 anions held by soil solids.

a) Effect of soil reaction. It has been widely shown that heavy liming and molybdenum application to molybdenum deficient soils frequently bring about the same improvementin yield (Kline, 1954, 1955; Anonymous, 1949; Plant, 1950; Askew, et. al. 1958; Botting, 1955; Clarke, et. al. 1957).

Mulder (1954), using Aspergillus niger, showed molybdenum in the soil to be more available with the medium at pH 6-8 than at pH 5 or 2. Lewis (1943) found that on the "teart" soils of Somerset where toxicity occurred in animals the pH was found over 7. When the pH was under 7 no toxic effect occurred, even when total molybdenum in the soil was as high as 33 ppm. It is true that molybdenum alone of the trace elements becomes more available in the soil with increasing pH. Jones (1957) shook soil with molybdate solutions at various pH levels and found that the amount of this element in the aqueous phase after shaking increased sharply from pH 4.5 to 7.2. Below pH 4.5 the amount of molybdenum in the solution was extremely low.

b) Fixation of molybdenum by soils. Fialdes (1954), stated that in the course of weathering molybdenum is released from the primary mineral to a more available state, only to be once more trapped at a later stage by fixation caused by clay minerals, amorphous hydrous oxides of Al, Fe, Ti, goethite and anatase. Wells (1956), in his study on molybdenum fixation, suggested that at the first stage of soil formation the soil has a high anion-exchange capacity and molybdate derived from the basalt is held in exchangeable form. With age, the hydrous iron oxides crystallize, and molybdenum becomes occluded and again unavailable. Thus, from this view, it should be concluded that young soils are likely to have higher available molybdenum than the mature soils. Stout, et. al. (1951), showed that molybdate was fixed by

hydrated halloysite and that much could be released by moderately alkaline solutions. This fixation of molybdenum by clay minerals in acid condition is further indicated by the finding of Barshad (1951a) that molybdenum was adsorbed by halloysite, kaolinite, and clay colloids up to pH 7.5, and no molybdenum was adsorbed above this pH. In their work, Robinson and Edgington (1954) also suggested a connection between abundance of free hydrated iron oxides and molybdenum availability, and the possibility of iron concretions locking up molybdenum in an entirely unavailable form.

Much of the work concerning molybdenum fixation in the soil system has been done by L. H. P. Jones (1957), who was able to demonstrate clearly that hydrated ferric oxide is largely responsible for molybdenum fixation. The adsorption took place at low pH and the amount adsorbed decreased with increasing pH. From this view he suggested that molybdenum, in ferruginous soils, is held on the surface of colloidal ferric oxides as the molybdate anion, which can be replaced by hydroxyl ions. It can be seed clearly that this work fits with the effect of lime on the availability of molybdenum to plants on some acidic, ferruginous soils. He also showed that aluminum and other clay minerals have a strong adsorption capacity for molybdate, and the order is: hydrous ferric oxide > aluminum oxide > halloysite > nontronite > kaolinite. Williams and Moore (1952) found that molybdenum uptake by Algerian oats in their experiment could be related mathematically to soil

pH and the amount of iron (soluble in 6N HCl) in the soil. They arrived at a regression equation of the form:

$$\text{Log } 100 \text{ Mo} = a\,pH - bFe + C$$

The authors also suggested that availability of molybdenum may depend on that of the MoO_4 ion through an adsorption process by free iron oxides. This suggestion agrees with that of Wells (1956) who used the term "retention" instead of "adsorption."

Molybdenum Content of Soils and Plants and Its Toxicity to Animals

In general, the molybdenum content of any plant is very low compared to that of other trace elements such as boron, copper, manganese and zinc. The range in ppm. of this element found in the plants is from 0.00 to more than 1,000 ppm., at which level there is no sign of toxicity to the plant itself (Hallsworth, 1958).

Evans, et. al. (1951) recorded the content of this element in lucerne as ranging from 0.1 to 1.4 ppm. on soils with an average content of the element ranging from 0.8 to 3.3 ppm. Fujimoto and Sherman (1951) found the content of this element in vegetation grown on Hawaiian soils to range from 0.0 to 2.5 ppm. They found the content in the soil to be very high and there was no correlation between the amount in the plants and total molybdenum in the soil. Cited by Fujimoto and Sherman (1951), Bertrand reported that leguminous plants which had a high content of molybdenum were found growing on soils with a molybdenum content ranging from 4.3 to 6.9 ppm. Ferguson, et. al. (1943), quoting Brenchley (1943), reported

the content of molybdenum in many plants as: wild white clover, 0.0095%; wild red clover, 0.089%; Yorkshire fog, 0.0069%; and perrennial rye grass, 0.0011%.

A comprehensive study on the relative uptake of molybdenum by legumes and non-legumes has been made by Barshad (1948, 1951a, b), who confirmed the results of Lewis (1943), that, in general, legumes take up more molybdenum from soil when available molybdenum is high than do non-legumes. Hewitt and Jones (1948), using sand culture, found a higher content of molybdenum in legumes than in the grasses, both grown under the same conditions. A pasture seeded to legumes thus can be expected to have a higher molybdenum content in the forages than one seeded to grasses. This is confirmed further by a conclusion made by Barshad (1948), that leguminosae has a higher requirement for molybdenum and thus a greater capacity to accumulate this element in its stems and leaves.

Forage which caused sickness among cattle was reported by Ferguson, et. al. (1943) to contain 14 ppm. molybdenum, and where the content was lower than 6 ppm. in pastures scouring was unknown. Muir (1941) was probably the first to state that the intake of excessive amounts of molybdenum was the direct cause of "teart" or scouring disease affecting animals. Britton and Goss (1946) found the molybdenum content of alfalfa to be very high and this was the cause of nutritional problems with cattle in California. Their analyses showed 6 to 36 ppm. molybdenum on dry weight

basis. Also, in order to prove that high amounts of this element caused the disorder, they demonstrated the development of symptoms of nutritional imbalance in a 6-month old heifer with 7 months by feeding 5 grams of sodium molybdate to the animal daily. When feeding of molybdenum was discontinued the affected calf recovered rapidly. Cunningham, H. M. et. al. (1953) reported the toxic herbage found in the Swan River Valley of Manitoba contained up to 25.6 ppm. Mo while pasture samples from other areas where the disease was not known contained less than 1.9 ppm.

Molybdenum Vs. Copper in Relation to Animal Nutrition

Cunningham, I. J. (1954) classified the molybdenum contents of pasture species in New Zealand in three classes: below 3 ppm. being normal; 3 to 10 ppm. being moderate; and above 10 ppm. being high. He pointed out that between 3 to 10 ppm. molybdenum a harmful effect can occur anytime when copper is low, and that a level above 10 ppm. can be harmful even when copper is normal. In 1956 he stated that pastures containing 1 ppm. molybdenum or less grew well and appeared normal and healthy, thus indicating that a molybdenum content higher than 1 ppm. in pasture crops is unnecessary; 3 ppm. was regarded as the critical level, especially when the copper content in the herbage was low.

Cunningham, et. al. (1956) reported that copper deficiency in stock, not complicated by molybdenum, has occurred in areas where the pasture

contained 3.5 ppm. of copper or less. The deficiency induced by excess molybdenum has been found on pastures containing more than 5 ppm. copper. Based on these findings he concluded that less than 5 ppm. copper in the herbage was regarded as a deficiency level, and interfering factors like excess molybdenum could readily induce copper deficiency symptoms in stock. However, the criteria used in this classification of the levels of copper in herbages in regard to animal nutrition differs slightly from one condition to another. Piper, et. al. (1951) classified forages containing copper less than 3 ppm. as deficient, 3 to 5 ppm. as marginal, and 7 to 12 ppm. as normal.

Molybdenum Content of Pasture Species in Relation to Climatic Factors

Barshad (1948) found that the variation in molybdenum content among different pasture species depended on the molybdenum which was water soluble in the soil, the time of the year the plant was grown, the age of the plant, and the plant part sampled. He found, in general, the older leaves tended to be higher in molybdenum than the young leaves. Different species also have different capacities for absorbing the element. Lewis (1943) found the clovers (Trifolium spp.) and the grass Yorkshire fog (Holocus lanatus) growing on a molybdenum rich soil contained much more molybdenum than other grasses growing in the same pastures. The level of molybdenum in the pasture crops in general slightly changed from season to season and year to year. Barshad (1951a) found that under California climate the molybdenum

content of ladino clover increased with age of the plant and was highest during the period of slow growth (fall and winter). He explained that a more rapid growth seemed to bring about a dilution of the molybdenum concentration in the plants during the spring and summer seasons. This finding is somewhat similar to that of Kretschmer, et. al. (1956) who discovered the molybdenum content in herbage grown on Everglades soils was higher in both fall and late spring than in the spring. He explained that the fall months gave a slower rate of growth of the plants. The late fall samples also gave him a high value of molybdenum due to the rate of growth reduced by plant maturation. Considering the copper content along with molybdenum, he found also that the average Cu/Mo ratios in the plants under study were higher in the spring due to the higher uptake of copper and dilution effect of growth on molybdenum. In the fall, copper seemed to be taken up less and molybdenum was "condensed" in the tissue due to the slower growth of the plant, thus bringing down the Cu/Mo ratio. The findings of both Barshad and Kretschmer are somewhat contradictory to that of Cunningham, I. J. et. al. (1956) who found the highest molybdenum content in herbages of New Zealand pastures in the spring due to the flush of heavy growth of the plants.

Effect of Phosphate on Molybdenum Uptake by Plants

The earlier workers in the field of molybdenum-phosphate interactions (Lewis, 1943; Anderson and Oertel, 1946) reported an enhancing effect of

phosphate ions on the uptake of molybdenum by legumes. Anderson and Oertel (1946) noted that sodium molybdate application to lucerne on a soil that had previously received 56 pounds superphosphate per acre increased significantly the molybdenum uptake of the crop. Lewis (1943) reported that the molybdenum content of herbage grown one year subsequent to an application of a nitrogen-phosphorus fertilizer showed an increase over the control treatment.

Further evidence for this interaction was provided by Stout, et. al. (1951) who found that increased phosphate levels in soil receiving one pound per acre of molybdenum increased the molybdenum content in plants, sometimes as much as 10-fold, over the molybdenum treatment alone. The increase in molybdenum content was also shown in water culture experiments, and the results obtained indicated a linear correlation between the amount of molybdenum taken up by the plant and the concentration of phosphate in the solution.

Similar results on the enhancing effect of phosphate on molybdenum uptake were obtained by Barshad (1951b), who found further that the effect was greater in acid than in alkaline soils. The mechanism of this enhancement effect is still not clearly understood. However, Stout, et. al. (1951) has suggested that the increased phosphate level may result in phosphate replacing molybdate on the anion exchange, thus releasing more molybdate into the soil solution. Another possible explanation is that the two elements

form a complex phosphomolybdate anion which is adsorbed more readily by the plant than the molybdate alone.

Cullen (1954), reported that improvement in yield of clover was much higher when phosphate was applied with molybdenum than when phosphate was applied alone. In 1956 he reported further that the application of molybdenum improved the efficiency of phosphate fertilizers for grasslands. This evidence further supports the mutually beneficial effects of the phosphate-molybdate interaction.

Effect of Sulfate on Molybdenum Uptake by Plants

The relationship of molybdate to sulfate in plant nutrition lies in the fact that sulfate depresses the uptake of molybdenum by plants. Stout, et. al. (1951b) showed that application of gypsum depressed the molybdenum content in peas. Also they found that, in addition to this depression effect, sulfate tended to cause retention of adsorbed molybdenum by roots of tomato plants at the expense of the tops when given small amounts of molybdenum over 24 hours without any effect on yield. Plant (1953, 1955) observed, however, that the effects of gypsum varied with soil type. In some soils he found an increase in molybdenum in the plant when sulfate was applied. This variation leads to unpredictable results in the effect of sulfate on molybdenum uptake which one might not suspect. Working with solution cultures Stout, et. al. (1951) found that, following an adsorption period of 41 hours, stem tissue of tomato plants contained 10.7 ppm. of molybdenum When sulfate was added the

molybdenum content dropped to only 2 2 ppb. Similar results were obtained for leaf and root tissues. This depressing effect of sulfate on molybdenum uptake can be further confirmed by the work of Reisenauer (1963), who found the molybdenum content of pea vines decreased appreciably upon the addition of sulfate at 20, 60, or 180 lbs. per acre. The depressing effect of sulfate was described by Barshad (1951b) and Stout, et. al. (1951) to be the result of direct competition between the molybdate and sulfate anions for adsorption sites on the roots.

MATERIALS AND METHODS

PHASE I

Seasonal Variation in Molybdenum Content of Pasture Species

A) Method of Collecting Data

The materials used in this experiment were plant samples representing six native pasture species collected from five different locations on the island of Hawaii. These samples represented only the above-ground portion including leaves, stem and sheath of grasses. Sampling sites were protected from interference by grazing animals by means of setting up cages. Samples were collected once every three or four months throughout the entire period of approximately four years and collected at the same time for all locations. The first sampling was made in February, 1959, and the last sampling in September, 1963. Within this interval the total number of samplings made at each location was 15. After each collection the samples were oven-dried at 70° C for 2-3 days, ground in a macro-willey mill and stored in stoppered bottles for subsequent chemical analysis for molybdenum.

Data concerning rainfall, elevation, and soil type were obtained from the ranchers.

B) Description of Locations

Description of the locations where pasture samples were collected are as follows:

1) Ohaikea Vee

This site is located on Kapapala loam soil. The soil belongs to the Olinda family of the Latosolic Brown Forest great soil group and is a sloping phase (8 to 25 percent; Cline, et. al. 1955). These pastures produce good quality forage with paspalum and rattail grass as the dominant species in the area. Average annual rainfall (1960-62) was 28.59 inches. The elevation is 3,460 feet and the area of this soil type consists of 3,729 acres

2) Pawaena

The soil type at this location is almost the same as at Ohaikea Vee. Kapapala loam and Kapapala fine sandy loam also belong to the Olinda family of the Latosolic Brown Forest great soil group and are gently sloping (3-15 percent slopes). These soils are more shallow than at Ohaikea Vee and are of more recent origin, developing from recent volcanic ash that originated at Kilauea Crater (Cline, et. al. 1955). Average rainfall for 1960-62 was 49.18 inches per year. Elevation of the total area ranges from 2,100 feet to 3,460 feet with an average in the middle of 2,800 feet. The main pasture crop in the area is rattail and the carrying capacity on this land is slightly less than that of Ohaikea Vee The total area consists of 2,462 acres.

3) Pahala

This area is located between Pahala and Punaluu with a deep mellow soil on gentle slopes. The soil is a Reddish Prairie of the Pahala family (Cline, et. al. 1955) and is quite productive because it has a deeper A_1 horizon and a

somewhat higher water-holding capacity. The area consists of 4,667 acres of canefield and pasture land. Quality of forage is very high and the amount produced also is relatively high, resulting in a carrying capacity of somewhat less than 4 acres per head.

Elevation is only 850 feet and the average rainfall is 37.26 inches per year. Kikuyu grass was the representative pasture sample collected in this area.

4) Haau

The soil type of this area is Moaula silty clay loam, gently sloping phase. It belongs to the Kapoho family in the Humic Latosol great soil group (Cline, et. al. 1955). The pastures produced are somewhat low in quality. However, rainfall is very high in this region, the average of three years (1960-62) being 72.24 inches per year which is the highest at all five locations studied. This soil is the youngest in the Humic Latosol group and has a dark reddish brown color and the pH is quite acid. Total area of this soil type is 1,104 acres with an elevation of about 1,250 feet Pangola grass was the representative pasture species at the sampling site on this area.

5) Shipman

Pastures in this area produce a dense stand of grasses which are used for grazing. The soil type is Kamaoa silty clay loam, sloping phase, which belongs to the Naalehu family in the Reddish Prairie great soil group (Cline, et. al. 1955). It is characterized by a brown A_1 horizon that rests on

yellowish-red or reddish-brown silty clay B horizons The soil is slightly acid throughout the solum. Pastures are normally dominated by Bermuda grass which tends to form a one grass sod. They are excellent for fattening cattle and the soil of the location is considered one of the best in the Hawaiian Islands for grazing. Elevation of the location is 1,660 feet and the three-year average annual rainfall is 34.22 inches Kikuyu and Desmodium uncinatum were the pasture samples from this location.

PHASE II

A Preliminary Survey of the Molybdenum and Copper Status of Some Pasture Species and Associated Soils on the Island of Hawaii

A) Soil and Plant Materials

The materials involved in this phase were 27 surface soil samples (0-6 inches deep) and 34 pasture plant samples, both collected on September 16-18, 1963; mostly from representative rangelands on the Island of Hawaii. Twenty-seven locations were selected for the survey. Twenty-three of these are on seven ranches and four are on a University experimental farm. Sampling sites were selected so that they represented the vast diversity of climatic conditions as elevation, soil type, rainfall, soil reaction as well as types of topography. At least one plant species was sampled at each soil site in order to obtain information on soil plant interrelationships. Many plant species were sometimes collected at the same site in order to permit a study of variations between plant species in accumulating copper and molybdenum taken up from the same soil. At the same time one main plant species, Kikuyu grass, was collected from as many soil sites as possible in order to evaluate the comparative molybdenum and copper status among different locations and conditions. The pasture species collected were:

1) Kikuyu grass: Pennisetum clandestinum
2) Guinea grass: Panicum maximum Jacq.

3) Rattail grass: Sporobolus capensis (willd) Kunth

4) Dayflower or Wandering Jew: Commelina diffusa Brown f.

5) Trefoil: Lotus spp.

6) White Clover or Dutch White Clover: Trifolium repens

7) Orchard grass: Dactylis glomerata

8) Sweet Vernal: Anthoxanthum adoratum

9) Mesquite grass: Holcus lanatus L.

10) Bermuda grass: Cynodon dactylon (1.) Pers.

B) Description of Locations

The names and description of 27 soil sites regarding their classification, rainfall, and elevations are presented in Table 1. Data pertaining to rainfall and elevation were obtained from the ranchers.

C) Methods of Sampling and Preparation of Samples

1) Soils

All surface soils were carefully dug with a shovel or soil sampling core, precluding any undecomposed organic materials and were put in polyethylene bags. The bags were kept tightly closed to prevent moisture loss and soil pH was immediately determined by the glass electrode technique (1:1 soil-water). Then they were air-dried and stored in tightly closed bottles for subsequent chemical analysis for total copper, total molybdenum and water extractable molybdenum

TABLE 1. DESCRIPTION OF LOCATIONS SELECTED AS SAMPLING SITES FOR SOIL AND PLANT SAMPLES. (ISLAND OF HAWAII)

Location	Elevation (feet)	Annual Rainfall (inches)	Great Soil Group [1/]	Soil Family [2/]
KUKAIAU RANCH				
1. Apoopuaa (Res. Ridge)	4500	45	L. B. F.	Hanipoe
2. Apoopuaa (Flat above)	4500	45	L. B. F.	Hanipoe
3. Puu Ulaula (Aa Flow)	7000	40	L. B. F.	Hanipoe
4. Coffee IA	3000	65	H. L.	Ookala
5. Hale Piula	5000	40	L. B. F.	Hanipoe
6. Holuokawaii	3800	35	L. B. F.	Maile
7. Iolehaehae (makai)	4500	50	L. B. F.	Hanipoe
8. Iolehaehae (mauka)	7500	35	L. B. F.	Hanipoe
KEAHOU RANCH				
9. Puu laa laa	5300	60	L. B. F.	Olinda
10. House Paddock	4500	50	Regosol	Manu
HORSESHOE I RANCH				
11. Kanakamilae	3600	60	L. B. F.	Maile
12. Nahuina	4400	35	L. B. F.	Maile
13. Hind	2600	60	H. H. L.	Kealakekua
14. Holokalele	2600	60	H. H. L.	Kealakekua
15. South New Field	5200	25	L. B. F.	Hanipoe
16. Waipio	5000	25	L. B. F.	Hanipoe

TABLE 1. DESCRIPTION OF LOCATIONS SELECTED AS SAMPLING SITES FOR SOIL AND PLANT SAMPLES. (ISLAND OF HAWAII)

(Continued)

Location	Elevation (feet)	Annual Rainfall (inches)	Great Soil[1/] Group	Soil[2/] Family
17. JOHN SOLOMON RANCH	-	-	L. B. F.	Maile
18. LUKE RANCH	75	15-20	L. H. L.	Kohala
19. KAHUA RANCH	3000	70	R. P.	Waimea
WAIMEA EXPERIMENTAL FARM				
20. Mud Lane A	2500	80-90	L. B. F.	Maile
21. Mud Lane B	2500	80-90	L. B. F.	Maile
22. Pukalani A	2600	35-40	R. P.	Waimea
23. Pukalani B	2600	35-40	R. P.	Waimea
PARKER RANCH				
24. Puunohu II 1	3500	25	R. P.	Waimea
25. Puunohu II 2	2500	25	R. P.	Waimea
26. Mud Lane Section	2500	80-90	L. B. F.	Maile
27. Kohala Section	-	-	L. B. F.	Maile

[1/] Based on Cline et. al. (1955): L. B. F. - Latosolic Brown Forest; H. L. - Humic Latosol; H. H. L. - Hydrol Humic Latosol; L. H. L. - Low Humic Latosol; R. P. - Reddish Prairie.

[2/] Based on Cline et. al. (1955).

2) Plants

All pasture plant species were collected carefully so that soil particles and dry leaves were precluded from the samples. The collected plant samples were then put in paper bags and the following parts were separated and prepared for chemical analysis for copper and molybdenum.

a) Kikuyu grass - top 2-1/2 inches measured from the spindle, most of dry leaves were discarded.

b) Trefoil - the compound leaves, 5 inches long measured from the tip of each branch.

c) Rattail grass - all green leaves and sheath 8 inches long, dead stems discarded.

d) Mesquite grass - all green leaves, stem discarded.

e) Sweet Vernal grass - all green leaves, stem discarded.

f) Wandering Jew - leaves and petioles.

g) Guinea Grass - all green leaves, stem discarded.

h) White clover - leaves and petioles.

i) Orchard grass - all green leaves, stem and sheath discarded.

j) Bermuda grass - leaves and sheath.

After oven drying at 70° C for 3 days, the selected plant parts were ground in a micro-wiley mill with a 20-mesh stainless steel screen and stored in stoppered bottles. The ground materials were subsequently subjected to analysis for copper and molybdenum.

PHASE III

Molybdenum Uptake by Plants as Affected by Molybdate, Phosphate, and Sulfate Fertilization

A) Description of Soils Used in Greenhouse Study

Surface soil samples from Waimea Experimental Farm and Kahua Ranch were sent to the University of Hawaii for the purpose of this study. Four soils were used in the study, two being taken from Mud Lane and one from the Pukalani section of the Farm area. The last soil was taken from Kahua Ranch. The names of the locations were used to identify the soils. The designated names of the four soils are: Mud Lane A soil, Mud Lane B soil, Pukalani soil, and Kahua soil.

1) Mud Lane A soil

This soil belongs to the Maile family in the Latosolic Brown Forest group. The soil has a dark-reddish-brown A_1 horizon and is relatively high in organic matter. It is a young soil on rapidly weathering materials consisting largely of iron oxide and aluminum in the mineral fraction. The soil pH at the surface is 5.8 which is the lowest of the four soils used in this study. The chemical properties are presented in Table 2. It has a medium crumb structure; very friable when moist; non-plastic when wet; and has numerous roots. It also has a high moisture holding capacity. Rainfall in this area is about 85 inches.

TABLE 2. SOME CHEMICAL PROPERTIES OF THE 4 SOILS USED IN GREENHOUSE STUDY[1/]

Soil	C. E. C. m. e./100 gm.	pH	Exch. Cations (m.e./100 gm.) Ca	Mg	K	Na	Base Sat. %	Ca Sat. %	Water Extr. Mo. ppm.	Total Mo. ppm.	Avail. P. 0.02N H_2SO_4 ppm.	Degree of Mo Fixation [2/]
Mud Lane A	98.0	5.8	8.2	5.19	.94	.59	15.0	8.36	.006	5.0	4	Very High
Mud Lane B	84.1	6.0	18.7	4.14	.77	.60	28.8	22.3	.013	4.8	13	High
Pukalani	65.7	6.9	22.0	6.10	3.20	.58	48.6	33.5	.036	2.9	660	Moderate
Kahua	51.2	6.2	32.9	5.20	4.20	.57	93.7	64.3	.028	3.8	148	Moderately High

[1/] Expressed in O. D. Basis.

[2/] Referred to Figure 1.

2) Mud Lane B soil

This soil belongs to the same family and group as Mud Lane A soil. The color of the surface soil and physical properties are very similar to those of Mud Lane A soil. However, the soil has slightly different chemical properties when compared with Mud Lane A soil (Table 2). Rainfall in the area where the sample was taken is about 85 inches.

3) Pukalani soil

This soil belongs to the Waimea family in the Reddish Prairie great soil group. It is characterized by a thick, dark-colored A_1 horizon with a stony phase. The soil is very dusty when dried and much lighter in color than when it is wet. It provides a good medium for plant growth with a tremendous surface area due to its fine texture. This soil is much higher in bases phosphate than the Latosolic Brown Forest soils. The chemical properties in regard to the greenhouse study are presented in Table 2. Rainfall in the area is about 20 inches.

4) Kahua soil

This soil has developed in a moderate annual rainfall area (25 inches). It belongs to the Waimea family in the Reddish Prairie great soil group. The physical properties are similar to the Mud Lane soils but differ slightly in that it has a lower water holding capacity and greater porosity. The base status is very high as is calcium saturation. Chemical properties of this soil are presented in Table 2.

B) Preparation of Soils

The soils were passed through a 1 cm. aluminum screen to preclude stones, gravels, and large roots. The screened soils then were mixed thoroughly to get a uniform growth medium.

C) Fertilizer Materials

All analytical reagents were used in this study. They were:

$Na_2SO_4 \cdot 10H_2O$ as sulfate source

$Na_2MoO_4 \cdot 2H_2O$ as molybdenum source

H_3PO_4 as phosphorus source

The pots had a diameter of 4.125 inches. This was equivalent to 13.461 square inches. Fertilizer application rates were calculated on a surface area basis.

D) Rates of Application

Three rates of application, 0, 250, and 500 lb/A, were assigned to phosphate, calculated as elemental P Two rates, 0 and 2 lb/A, were assigned to molybdenum based on elemental Mo, and two rates, 0 and 100 lb/A, were assigned to sulfate as SO_4. Calculated on a milligram basis, the P_{250} and P_{500} levels were equivalent to 0.76 mg. and 1.52 mg. H_3PO_4 per pot, respectively. The 2 lb/A rate of Mo was equivalent to 4.9 mg. of $Na_2MoO_4 \cdot 2H_2O$ per pot. Sulfate was applied all at the rate of 339 mg. of $Na_2SO_4 \cdot 10H_2O$ per pot. The nutrients were prepared as solutions and applied from a pipette directly to the soil. The soil was then mixed thoroughly and potted in cans

lined with polyethylene bags.

Fertilizer treatments of P, SO_4, and Mo were designated as follows:

P_0 - no P applied

P_{250} - P at 250 lb/A

P_{500} - P at 500 lb/A

No Mo - no molybdenum applied

Mo - applied at 2 lb/A

No S - no sulfur applied

S - applied at 100 lb/A

E) Experimental

The experiment was carried out as a 2(Mo) x 2 (SO_4) x 3(P) factorial in a randomized complete block design with four replications. Each soil was considered as a separate identity and thus the soils were not regarded as variates. The amount of each soil used per pot was: Mud Lane A soil, 1,070 gm. air-dried or 686 gm. O.D. basis; Mud Lane B soil, 1,080 gm. air-dried or 656 gm. O.D. basis; Pukalani soil, 1,100 gm. air-dried or 973 gm. O.D. basis; and Kahua soil, 1,257 gm. air-dried or 927 gm. O.D. basis. Difference in weight of soil used was due to difference in volume weight of the soils and differences in moisture content at the time of potting. Desmodium tortuosum, a legume, was selected as a test plant because of its higher requirement for Mo compared to grasses. The seed used was a mixture of plant introductions 5403, 5515, and 5042. It was first scarified by soaking in concentrated

sulfuric acid for 25 minutes and then in running tap water for another three hours. Then the seed was washed with distilled water and planted directly into the pots using 15-20 seeds per pot. An inoculation was made in order to insure a good supply of nitrogen by applying 5 ml. of a thick suspension of rhizobium (Desmodium spp.) to each pot. The seedlings were allowed to grow for one week and then thinned to four plants per pot by hand. Random arrangement of all pots on the bench in the greenhouse was made at time of planting and rearranged three weeks after planting to aboid micro-environmental effect. Distilled water was used for watering and soil moisture was maintained at 70 percent of field capacity throughout the period of the experiment. After 6 weeks, the plants were harvested by cutting approximately 1 cm. above the soil level. Samples were oven-dried at 70° C for 3 days and then weighed to obtain yield data. The dried plant materials were then ground in a willey mill (stainless steel) using a 20-mesh screen and stored in a paper sack. Samples were re-dried prior to chemical analysis for molybdenum, phosphorus, and sulfur.

Due to inadequate plant tissue which resulted from poor growth of plants on Mud Lane A and Mud Lane B soils, replicate samples from these two soils were composited and duplicate analyses were made for each of the three elements. On the other two soils, Pukalani and Kahua, growth of Desmodium was good for every treatment, and this provided adequate sample for separate chemical analyses.

Before application of Mo to each soil it was felt that a fixation study of these soils should be carried out in order to get some idea of how much Mo would be available to the plants grown on each soil. This was done by shaking 10 gm. of soil with 100 ml. of sod. molybdate solution (containing 200 μg. Mo). After equilibrium, the suspension was filtered and a 10 ml. aliquot of filtrate used to determine Mo. The amount remaining in solution was expressed as a percentage of the total amount initially added. It was found that the fixation capacity of the 4 soils for Mo was in the order: Mud Lane A > Mud Lane B > Kahua > Pukalani (Figure 1). Mud Lane A soil fixed most of the Mo added; only 2.3 percent was found remaining in solution. Pukalani soil fixed the least with 52 percent of the Mo remaining in solution.

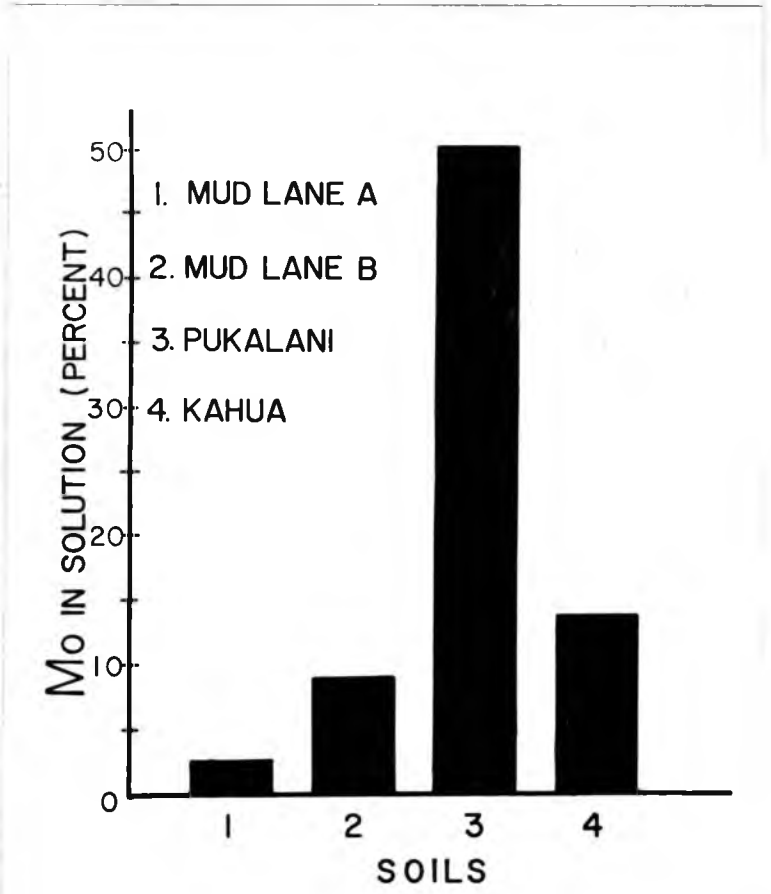

FIGURE 1. MOLYBDENUM FIXATION CAPACITY OF 4 SOILS USED IN GREENHOUSE STUDY. (Mo added = 200 μg. in 100 ml. water per 10 gm. soil)

PHASE IV

Availability of Molybdenum in Simplified Soil Systems as Influenced by pH of the System and by Phosphate and Sulfate Concentrations

A) Experimental Statements

Relationships between soil reaction and molybdenum availability have been extensively studied (Magee, 1933; Plant, 1950; Plant, 1951; Mulder, 1954; Anderson and Oertel, 1946; Barshad, 1951a; Stout, et. al., 1951). The influence of soil reaction on molybdenum availability is due mainly to the activity of hydroxyl ions in the solution. Increasing the pH of a soil increases the hydroxyl ion concentration and enhances anion exchange between molybdenum that is fixed or adsorbed by clays or soil colloids and hydroxyl anions (Stephens and Oertel, 1943). However, the extent to which the soil releases molybdate anions when the pH is increased depends also upon the nature of the soil, mainly its chemical composition. Halloysite has been shown by Stout, et. al. (1951) to be responsible for molybdenum fixation, particularly in acid media. Iron oxide, however, fixes molybdenum more strongly than other soil constituents (Wells, 1956; Jones, 1957). Aluminum also plays a major part in molybdenum fixation. Jones (1957) shaked molybdate with a solution of aluminum trichloride and obtained the formation of an insoluble aluminum molybdate compound. He suggested the formula to be 2 $(Al(H_2O)_4(OH)_4MoO_4$. Other clay minerals such as nontronite and kaolinite also fix molybdenum to some extent; the effect being less than that of aluminum oxide and iron oxide which

have the highest fixation capacity. Fixation capacity of the constituents mentioned will decrease when the pH of the system is increased.

The enhancing effect of phosphate on molybdenum uptake by plants has been noted and demonstrated by many workers in the field (Lewis, 1943; Anderson and Oertel, 1946; Stout, et. al., 1951; Barshad, 1951b; Cullen, 1954). Stout, et. al. (1951) suggested the possibility of anion exchange between molybdate and phosphate but put major emphasis on translocation of molybdenum as initiated and enhanced by phosphate when he worked with tomato plants in culture solution. Mulder (1954) demonstrated increases in available molybdenum in soils due to phosphate addition using the A. niger method. It is seen from the above findings that although major emphasis concerning increased molybdenum uptake due to increasing levels of phosphate has been placed on effects within the plant, anion exchange within the soil also may contribute significantly to increased molybdenum availability and hence to increased Mo in the plant.

Sulfate, on the other hand, depresses the uptake of molybdenum. The effect is attributed to direct competition between two anions of similar size and charge for adsorption sites on the roots (Stout, et. al., 1951). It is felt that if the anion exchange process between molybdate and phosphate holds and results in increased molybdenum in the soil solution, then this exchange process might also hold between molybdate and sulfate. At the present time the experimental work on this exchange mechanism is not conclusive. This experiment

was carried out, then, in an attempt to determine the mechanism of exchange between molybdate and sulfate anions. The experiment places major emphasis on demonstrating that increased molybdenum availability in soil can be a direct result of anion exchange whether either phosphate or sulfate concentration is increased.

This phase of the thesis was also aimed at a study of pH and molybdenum availability in soils using simplified soil systems. The relationship between pH and molybdate fixation (or availability in terms of the amount remaining in solution) in some soils of the Hawaiian Islands was determined, and also the pattern of the relationship which differs from soil to soil.

B) Description of Soils Studied

The soils used were an Akaka soil from the Island of Hawaii, a Naiwa surface soil from Molokai, and a Coral sand from Oahu. Descriptions of these soils are as follows:

1) Akaka soil

This soil has a prominent character in that it has a high fraction of amorphous materials which consist largely of hydrous oxides of aluminum and iron. The soil has an extractable aluminum content of 2,856 ppm. ($BaCl_2$ Method) and a pH of 5.00. The major clay minerals in this soil besides the amorphous materials are allophane (30 percent), gibbsite (25-35 percent), and magnetite (5-20 percent) (Tamura, et. al., 1953). The free iron oxide

content of this soil runs about 19.6 percent. Moist soil was used for the experiment.

2) Naiwa soil

This soil belongs to the Naiwa family of Ferruginous Humic Latosol group. The surface horizon is high in titanium oxide, 19.2 percent, and very high in iron oxide, 45.9 percent (Sherman, et. al., 1955). Minerals present in this soil are ilmenite, magnetite, a small amount of gibbsite, goethite, and halloysite (Silva, 1963). The amorphous fraction of this soil had been greatly reduced by air-drying of the sample. Exchangeable aluminum is much lower than that of Akaka soil. The pH of the air-dried sample is 4.3. This soil was obtained from Molokai Island.

3) Catano Sand

This sand has a very high carbonate content. The clay mineral fractions including iron oxide and alumina are almost absent. It was taken from a beach area on Oahu.

C) Experimental

1) Mo availability in soil solution as influenced by pH of the soil system

The experiment was carried out in duplicate as follows: 5 gm. samples (O.D. basis) of soil were weighed into a series of 250 ml. Erlenmeyer flasks. 100 ml. of distilled water containing 200 g of molybdenum and varying amounts of HCl and KOH were then added to each flask. Varying amounts of

HCl and KOH (both prepared from 2 N solution) were estimated from a few test samples in order to obtain different pH values of the soil suspensions varying from pH 2.0 to approximately 9.0 The flasks were shaken on a mechanical shaker for 1 hour, allowed to stand for 25 hours, and shaken again for another 1 hour. (From a preliminary study, this time span was found adequate for final equilibrium) The pH of the suspension was determined and then the suspension was filtered through Whatman No. 40 filter paper and 10 ml. of filtrate were taken for determination of molybdenum remaining in solution. For Naiwa soil it was found difficult to get a clear solution by filtration; thus, the suspension was centrifuged at 2,500 rpm. for 30 minutes and 10 ml. of clear supernatant solution was pipetted out for molybdenum determination.

2) Molybdenum availability in soil solution as affected by phosphate and sulfate concentrations

Five gm. (O.D. basis) samples of each soil material were placed in a series of 150 ml. beakers each containing 50 ml. distilled water. Then 2 ml. of 100 ppm. Mo solution (as sod. molybdate) were added to each beaker. This gave a 2 ppm. concentration in the final volume (100 ml.). In the experiment with phosphate, varying amounts of P stock solution (10,000 ppm.) prepared from potassium monobasic phosphate were added to give final concentrations of P in the soil suspension of 0, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, and 3000 ppm. The volume was adjusted to 90 ml. with distilled

water and the suspension was stirred frequently with a glass rod during a 48 hour standing period. (A preliminary experiment showed that this length of time was fully adequate for complete equilibrium). In order to permit a comparative study between different soils, the pH of the soil suspension for every soil and treatment was adjusted to one value. A pH of 5.9 was selected for this study because the soils would not be sufficiently acid to fix all the Mo, nor would they be alkaline enough to release too high an amount of Mo into solution. Thus, an adequate amount of Mo would be present in the solution for accurate analysis. Due to the two hydrogen atoms in potassium monobasic phosphate, the soil suspension tended to be more acidic with increasing levels of phosphate. This was corrected by adding a few drops of either 1 N or 6 N potassium hydroxide solution to the suspension, and pH was determined by glass electrode. At the end of the standing period the pH was checked again and adjusted, if necessary, to 5.9 ± 0.05. Then the suspension was diluted to 100 ml. volume (measured with a 100 ml. graduate cylinder) with distilled water and stirred vigorously for 2 more minutes and filtered through Whatman No. 40 filter paper. A 10 ml. portion of clear filtrate was taken for Mo determination. For the Naiwa soil which tended to yield a high amount of inorganic soil colloids, the solids were separated by centrifuging the soil suspension at 2,500 rpm. for 30 minutes.

For the sulfate experiment, the same procedure was followed except that the levels of sulfate used were 0, 100, 300, 500, 1000, 1500, 2000, 2500,

and 3000 ppm. SO_4. Calcium sulfate was used and this salt tended to increase the pH of the suspension on standing. However, the pH was adjusted to 5.9 ± 0.05 by adding a few drops of either 1 N or 3 N hydrochloric acid solution.

In Catano sand which contains a high amount of carbonate, the pH had to be adjusted more frequently and 12 N hydrochloric acid was used instead of 3 N in order to overcome the alkalinity developed from calcium carbonate.

The experiment was replicated twice and the average amount in μg. of Mo remaining in 10 ml. solution was then plotted against the concentrations of phosphate and sulfate.

Analytical Procedures

The following are the analytical procedures that were employed for this thesis problem.

1) pH was measured on 1:1 soil-water suspensions after a 24-hour equilibration period using the conventional glass electrode method.

2) C.E.C. was determined by using 1 N ammonium acetate adjusted to pH 7.0 as extractant. The method is described by Piper (1944).

3) Extractable calcium and magnesium. The ammonium acetate extract from (2) was analyzed for Ca and Mg by EDTA titration as described by Chapman & Pratt (1961).

4) Available phosphorus. Available phosphorus was determined by the HSPA modification of the Truog method. The soil was shaken with 0.02 N H_2SO_4 solution, and phosphorus determined by the chlorostannous-reduced

molybdophosphoric Blue Color Method in sulfuric system as outlined by Jackson (1960).

5) Plant molybdenum. Using a pilot sample, the ground plant material was weighed accurately into a porcelain crucible. A 0.5 gm. sample for a Mo content of 20 ppm. and a 3-7 gm. sample for a Mo content of less than 5 ppm. was normally adequate. The sample was dry-ashed in an oven at 600° C for at least 12 hours. The ash was taken up with 10 ml. double distilled water and 10 ml. 6.5 N HCl + $FeCl_3$ solution. After the ash was dissolved by gentle heating on the hot plate, the acid extract was filtered (Whatman No. 54) and the whole aliquot of filtrate used to determine molybdenum by stannous chloride-molybdothiocynate method as described by Johnson and Urlich (1959).

6) Plant copper. About 2 gm. of plant samples were weighed accurately into a 100 ml. Kjedahl flask. The sample was predigested overnight with 15 ml. conc. nitric acid and then digested at full heat (180° C) with 10 ml. of ternary acid mixture (Nitric: perchloric: sulfuric conc. acid mixture of the ratio 10:3:1). The final moist residue was taken up with 10 ml. 6 N hydrochloric acid and diluted to 50 ml. Usually a 20 ml. aliquot was transferred into a 60 ml. Squib seperatory funnel. Five ml. of 10 percent hydroxylalmine-hydrochloride solution was then added followed by the addition of 3 N sodium acetate solution until the pH of the solution was between 4.5 and 7.5. The solution was diluted to 40 ml. with double-distilled water, 5 ml. of

0.02 percent cuproine solution in isoamyl alcohol was added, and the funnel shaken 200 times by hand. After 15 minutes for phase separation, the lower aqueous layer was discarded and the clear purple alcohol layer was drawn into a photocell (Beckman DU Spectrophotometer). The color was read at 545 mμ and the reading compared to a standard curve prepared by adding varying amounts of copper from 0.0 to 30 μgm. into the seperatory funnels. The color persisted for four days without changing. Double-distilled water (pyrex) was used throughout the procedure including washing glassware and preparation of reagents.

7) Plant phosphorus. About 0.5 gm. of plant sample was weighed accurately into a 125 ml. Erlenmeyer flask and the sample digested with 10 ml. of 2:1 nitric-perchloric acid mixture until the residue was white. Before the perchloric acid became completely dry the digested residue was cooled and dissolved in 10 ml. 6 N HCl. The plant extract was diluted to 100 ml. and a 10 ml. aliquot was taken for phosphorus determination by "yellow color vanadomolybdate method" as described by Chapman and Pratt (1961).

8) Plant sulfur. A 10 ml. aliquot of the plant extract for phosphorus determination was transferred to a 25 ml. volumetric flask and analyzed for sulfur (total sulfur in plant) as sulfate by the Barium Chloride Turbidity Method as outlined by Kacar (1962).

9) "Total" soil molybdenum. Determination of total soil molybdenum has been subject to many modifications and many independent methods have

developed. This has caused a serious variation in results from laboratory to laboratory. The modifications have necessarily been made as a result of trying to avoid interference from the heavy metals vanadium, titanium, and chromium, and also in an attempt to arrive at the "total" fraction of this element in the soil. The method of Fujimoto and Sherman (1951), which was employed for several samples of Hawaiian soils about 13 years ago, gave very high values due to the inclusion of many interfering heavy metals through the use of a strong acid as soil extractant. Robinson, et. al. (1953) claimed that their method of using "alkaline separation" was more reliable and gave results in better agreement with the average values of the world. Perrin's method (1946) required too large a sample size for thorough fusion and did not work successfully with Hawaiian soils due to the high content of free oxides which are not attacked by HF. This tends to give a lower analytical value. For the purpose of this thesis, a method for the determination of "total" soil molybdenum was carefully worked out in the hope that it would give a accurate, reliable, and reproducible results, and would be essentially free from interference by Ti, V, and Cr. The method developed was quite successful but very tedious and time-consuming and is, therefore, not recommended for routine analysis of a large number of samples.

Extraction of soil by alkaline separation as described by Robinson (1948) was chosen with only slight modifications for the first part of the procedure. The color development section, being the second part, was based

on the formation of an amber-red molybdo-thiocyanate complex which can be extracted with a 1:1 mixture of carbontetrachloride and isoamyl alcohol. This part was adapted from the method of Johnson and Arkley (1954).

For analysis about 0.4 - 0.8 gm. of oven-dried soil which has been finely ground is accurately weighed in a platinum crucible, mixed with 5 gm. anhydrous sodium carbonate and fused for 1 hour and 20 minutes. The sample is first heated gently over a low Bunsen flame for 12 minutes with the lid on. Then the flame is increased to its hottest temperature for 5 minutes before changing to a Meeker burner. The crucible was then heated with a Meeker burner at 900° - 1000° C for 15 minutes, and the lid taken off and placed in a 400 ml. pyrex beaker containing 100 ml. water plus 3 ml. of 95 percent ethyl alcohol. The crucible was fused further for 50 minutes with constant swirling. This was necessary in order to get complete oxidation of the molten mass and a uniformly fused cake. Usually, swirling the crucible five times was found to be adequate to obtain a good thoroughly fused cake that would readily disintegrate in water. After the fusion was completed, as shown by quietness of the molten mass, the crucible was left to cool and then immersed in the beaker in which the lid had been placed. The crucible was left submerged overnight and then taken out with the lid. Trouble usually developed in bringing the residue on the inner side of the crucible into solution. This, however, was facilitated by means of tituration with a rubber policeman. This process was tedious and required about 20 to 40 minutes before

disintegration of the fused cake was complete. Then the crucible and lid were removed and a few drops of conc. sodium peroxide solution were added to insure a complete conversion of reduced forms of molybdenum to hexavalent state. The solution and residue were further digested on a hot plate at low temperature for 3 hours. In some instances, the residue still did not thoroughly disintegrate into a slurry. This was accomplished by transferring the residue to a mortar and crushing it until it formed a slurry and then it was transferred back to the beaker. The warm solution was iltered through a White Ribbon No. 589 filter paper using 30 ml. of warm 4 percent sodium carbonate solution for washing the precipitate. The washing and clear filtrate were combined, heated on the hot plate until the volume was about 15 ml., and then titrated to neutrality with 12 N HCl using litmus paper as indicator. The solution was then transferred to a 60 ml. Squib seperatory funnel and diluted to the 45 ml. mark with 10 ml. of 6.5 N hydrocholoric acid-ferric chloride solution and water. A 3 ml. aliquot of a 1:1 mixture of isoamyl alcohol and carbontetrachloride was added and the funnel shaken vigorously by hand 100 times. The organic phase was permitted to seperate for 15 minutes and discarded quantitatively. The following reagents were then added with the funnel shaken after each addition: 1 ml. of 15 percent citric acid, 2 drops of 30 percent sodium thiocyanate solution, 1 ml. of 40 percent stannous chloride in dilute hydrocholoric solution. This time, exactly 3 ml. of the extractant was added from a pipette and the funnel shaken

vigorously by hand 200 times. After allowing the mixture to stand for 1 hour, the colored organic layer was drawn into a spectrophotometer cell (Beckman DU Quartz) and the optical density measured at 470 mμ.

Before drawing the colored organic layer from the funnel any trace of water adhering to the inside wall of the funnel stem was eliminated by drying with tissue paper. In some soil samples a golden off-color yellowness developed and dominated completely the amber-red color of the molybdothiocyanate complex. To eliminate this interference the cells were placed in an electric oven at 100° C for 2 minutes, cooled, and transferred into the spectrophotometer. This heating effect not only eliminated the interference, but also eliminated any cloudiness in the organic layer which was occasionally caused by the presence of a trace of moisture. Evaporation effects and changes in color of the complex did not occur unless the cells were in the oven for more than 4 minutes. The color complex was stable for more than 8 hours. Double-distilled water (pyrex) was used throughout the procedure. A standard curve was prepared by adding 0, 5, 10, 15, 20, and 30 micro-gm. amounts of Mo as Na_2MoO_4 solution (prepared from MoO_3) to the platinum crucibles and then evaporating the solution to dryness. The crucibles were subjected to fusion and the same procedure followed as for the sample determinations.

10) Total soil copper. The method used included standard sodium carbonate fusion of the samples in platinum crucibles. Following dissolution of the fused cake, copper was determined colorimetrically by the Biquinaline method.

About 0.5 gm. of oven-dried soil which had been finely ground was accurately weighed in a platinum crucible and mixed with 3 gm. anhydrous sodium carbonate. The fusion technique was identical with that for molybdenum analysis. After 1 hour of fusion the fused cake and crucible were immersed in a 250 ml. beaker containing 120 ml. of 1.5 N hydrochloric acid. When the cake was completely disintegrated the lid and crucible were removed, and the solution was digested at near boiling for 1/2 hour. Ten ml. of 30 percent ammonium chloride solution was then added, and the solution was titrated to neutrality by the slow dropwise addition of ammonium hydroxide. The solution was stirred vigorously with a glass rod and then 3 ml. of ammonium hydroxide were added. Silica, iron, and alumina were then separated by filtration using a White Ribbon No. 589 filter paper Precautions were taken to avoid adsorption of copper by the colloids by allowing the precipitate to settle in the beaker first before filtering, and the supernatant solution was carefully decanted into the filtering funnel. After most of the clear solution had been filtered, then the precipitate was allowed to come into the funnel and was washed with several small lots of 5 percent ammonium chloride in 1 N ammonium hydroxide solution. The clear filtrate was then evaporated at low heat to drive off excess ammonia and reduce the volume down to approximately 35 ml. The solution was then diluted to 50 ml. and a 25 ml. aliquot taken for copper determination by the method described for plant copper analysis. Double distilled water (pyrex) was used throughout the procedure. The standard curve

was prepared by addint 0, 5, 10, 15, 20, and 30 gm. amounts of copper as copper sulfate solution to platinum crucibles and evaporating the solutions to dryness in an oven. The standard samples were then fused and treated the same as in sample determinations.

11) Water extractable Mo in soils. A 50 gm air-dried soil sample was placed in a 250 ml. Erlenmeyer flask with 200 ml. of distilled water and the flask shaken for 1 hour. The suspension was filtered under vacuum (Whatman No. 40) and the soil was removed from the filter paper and shaken with another 200 ml. distilled water. This process was repeated for a total of four extractions and the filtrate plus washings were all combined (about 900 ml.) and evaporated to dryness on the hot plate. The residue was treated with 10 ml. of a nitric-perchloric acid mixture (1:1) to remove organic matter and after perchloric was driven off the white residue was dissolved in 10 ml. of 6 N HCl. The solution was then transferred to a 60 ml. Squib seperatory funnel and molybdenum determined as described under plant molybdenum analysis.

Statistical Inferences

The results of the greenhouse study--yield data, molybdenum content, phosphorus content, sulfur content--were analyzed statistically by a factorial analysis of variance (Snedecor, 1961). Duncan's test of differences among means was applied (Duncan, 1955). Linear regression and correlation were used in Phase II to establish relationships between the two variables (Snedecor, 1961).

RESULTS AND DISCUSSION

PHASE I

Seasonal Variation in Molybdenum Content of Pasture Species

a) Seasonal variation as influenced by rainfall. In general the average Mo content of pasture forage was highest in Ohaikea Vee and lowest in Shipman. This could possibly be due to the difference in the molybdenum status of the soil of the two locations and also the plant species grown on them. The degree of fluctuation of molybdenum in pastures, again, was observed to be highest in Ohaikea Vee and lowest in Shipman. This high degree of fluctuation of molybdenum level in the pasture plants (paspalum and rattail) grown in Ohaikea Vee could certainly be the result of the high degree of rainfall fluctuation itself. The first peak of rainfall within the time studied occurred around March, 1960 and the molybdenum level was quite high (Figure 2). When the rainfall dropped in August, the same year, the level of Mo also seemed to have dropped. From September, 1960 to Arpil, 1961, the rainfall was quite uniformly distributed and was highest in January, 1961. Again, the Mo level went up and the peak occurred one month following the peak of rainfall. This relationship between rainfall and Mo level in Ohaikea Vee can further be seen distinctively in the years 1962 and 1963. The Mo level followed closely the rainfall fluctuation and its peak (4.30 ppm.) occurred almost at the same time as that of rainfall in the entire period

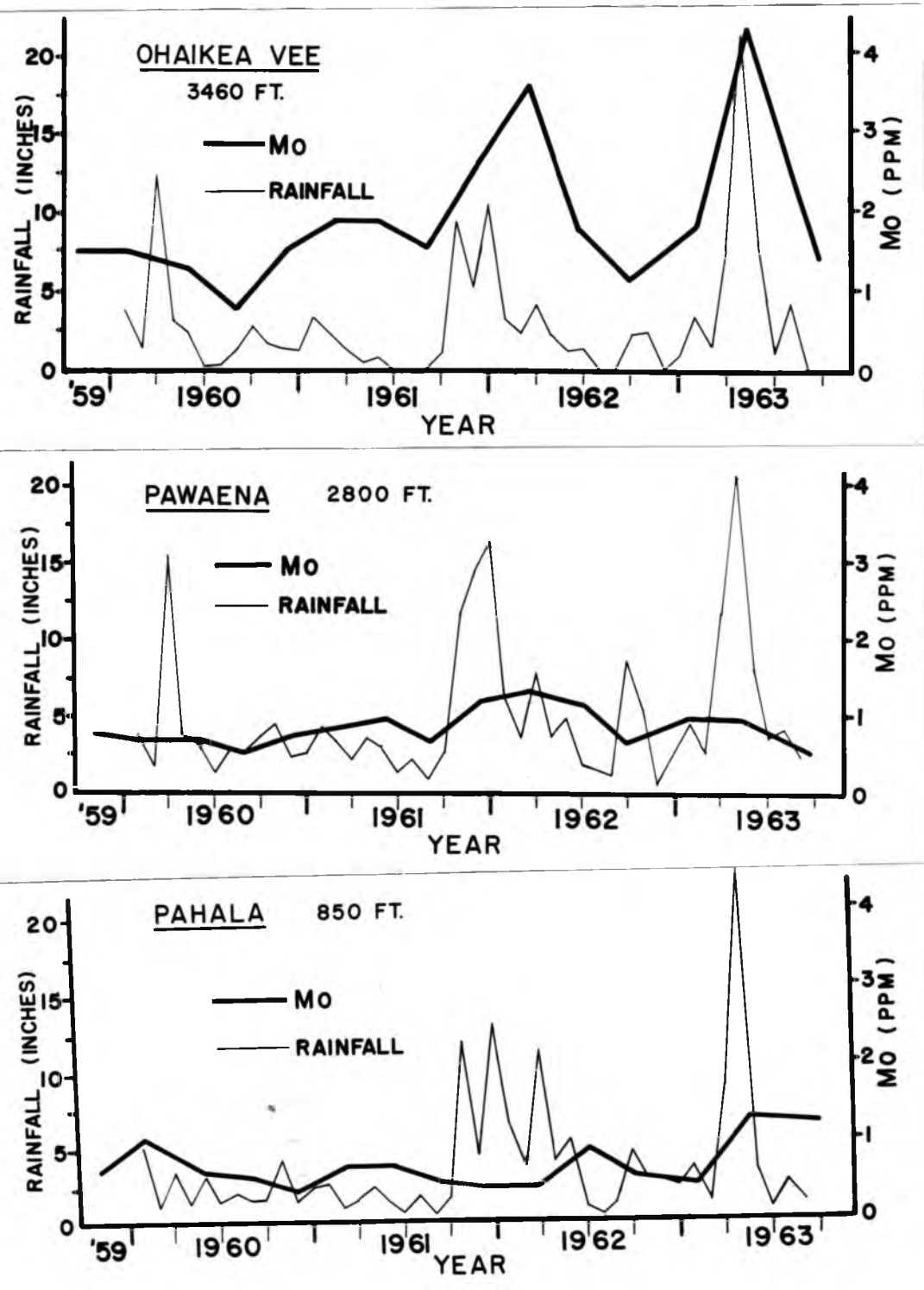

FIGURE 2. SEASONAL VARIATION IN MOLYBDENUM CONTENT OF PASTURE SPECIES GROWN ON 3 LOCATIONS WITH MONTHLY DISTRIBUTION OF RAINFALL.

studied. Thus, one can see how rainfall exerts a great influence on the level of Mo in pasture forages grown on this location. The increase of rainfall certainly increased Mo availability in the soil, resulting in higher uptake of Mo by the plant. It possibly also increased the growth status of the plant and adsorbing capacity of the roots for nutrient supplies including Mo.

The effect of rainfall on the Mo level of pasture plants grown in Pawaena was not distinct as in Ohaikea. However, the same trend was obtained, although the fluctuation of the Mo level was not high. The positions of various peaks of rainfall and Mo levels on these two locations were very much the same due to the close proximity of the two areas. Regarding the average Mo level in both pastures, Pawaena gave a lower average Mo content in plants and this could possibly be due to the lower Mo status in the soil.

In Pahala, the pattern of rainfall distribution throughout the entire period of four years was similar to those of the two locations previously discussed in that a high December peak and the highest April peak were obtained in 1961 and 1963, respectively. The level of Mo in plants in this location did not seem to follow the pattern of rainfall. However, the trend observed in 1962 and 1963 was quite similar to those of the other two locations in that the Mo peak in 1962 followed a few months after the rainfall began to drop off and in 1963 the highest level of Mo was found coinciding with that of rainfall around April or May (Figure 2). It seems that the Mo level of forage on this location was less dependent on the rainfall pattern than on Ohaikea Vee

and Pawaena. This might be explained by the fact that different species of plants have different capacities to absorb the elements from the soil and also due to the difference in sensitivity among the plants themselves to detect any small change of Mo availability in the soil solution. Kikuyu grass was the plant sample obtained from the area.

The average values of Mo content in plants at both Haau and Shipman were found to be very low as compared to the other three previously discussed areas (see Figure 3). The average Mo content of plants in Haau was 0.19 ppm. while that in Shipman was 0.18 ppm (Appendix I). It is to be observed from this study that the lower the average content of Mo in the pasture species the lower the degree of fluctuation There was almost no fluctuation at all in Haau where the rainfall was very high, the three-year average being 72.24 inches per year. The fluctuation of rainfall in this location was much less than in the others and this possibly caused the low fluctuation of Mo level in plants observed in this location. In Shipman, there was a greater fluctuation of Mo level than in Haau. However, the pattern of rainfall did not fit well with the pattern of Mo level as in the first two locations. It might be that because rainfall of these two locations were generally well distributed, the moisture level of the soils was maintained so high at all times that the availability of Mo reached its maximum in the soils. This might lead to a condition where the Mo content in plants was no longer a function of rainfall The variation of Mo level then may be a result of the

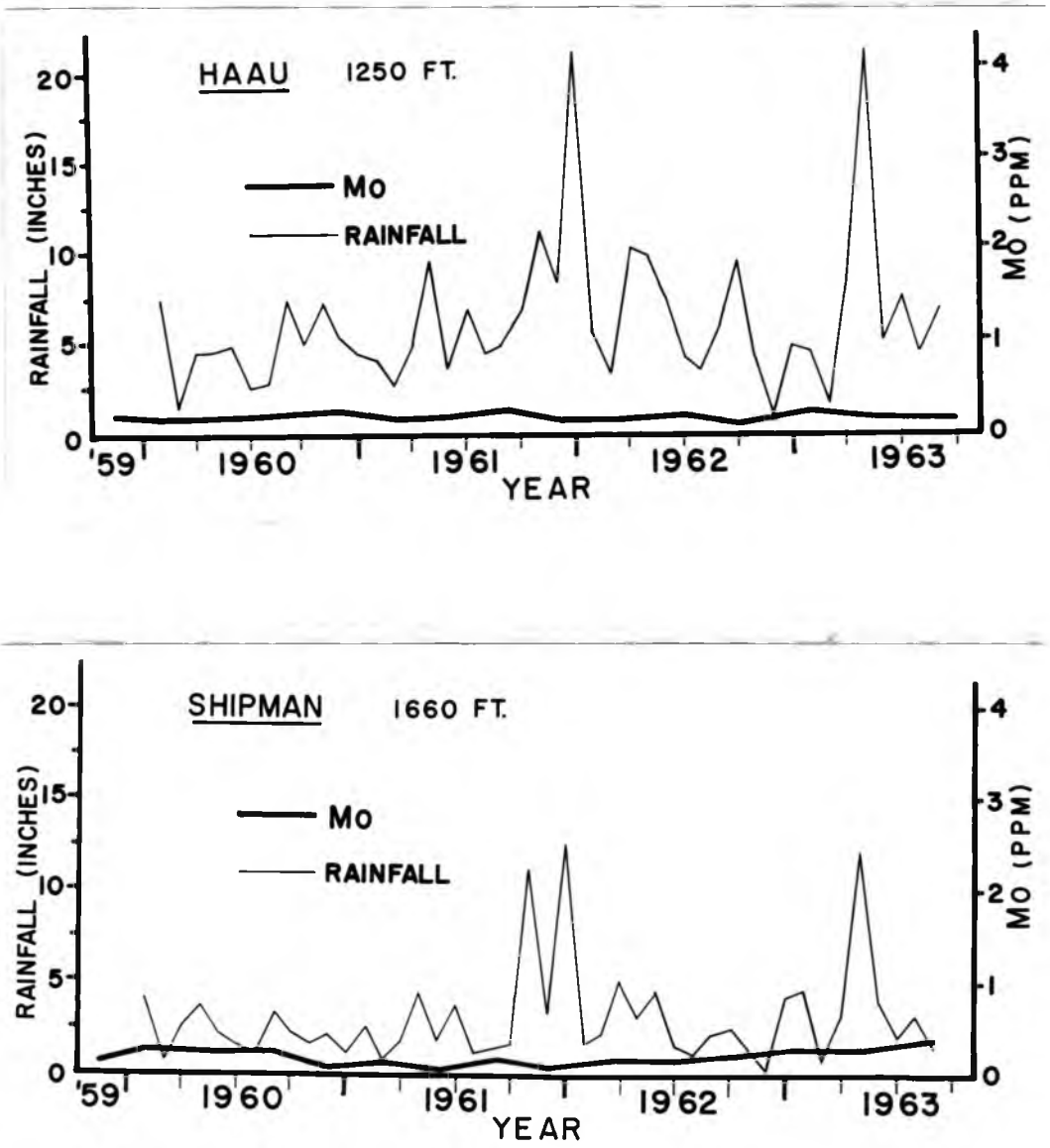

FIGURE 3. SEASONAL VARIATION IN MOLYBDENUM CONTENT OF PASTURE SPECIES GROWN ON 2 LOCATIONS WITH MONTHLY DISTRIBUTION OF RAINFALL.

growth of the plant itself (dilution effect) or the degree of maturation at particular sampling dates.

On the whole, rainfall played a great influence on the Mo content of plants in Ohaikea Vee and Pawaena. This is possibly due to the direct effect of rainfall on the availability of Mo in the soil solution and the capability of the plants themselves (rattail and paspalum) to absorb Mo according to the concentration of the element in the soils. Rainfall seemed to have some influence on Mo level of Kikuyu grass grown in Pahala and did not have any effect on plants grown in Haau and Shipman due to the more even distribution all through the years on these latter two locations. The average values of Mo in pasture plants on 5 locations fall in the order: Ohaikea Vee > Pawaena > Pahala > Haau > Shipman. The figures are 1.91, 0.82, 0.71, 0.19, 0.18 ppm., respectively (see Appendix I). However, the rainfall averages of 5 locations did not follow this same order The comparison of seasonal variation in Mo content of pasture species grown on 5 different locations is presented in Figure 4.

b) Great soil group and soil family in relation to the Mo levels of pastures. Considering the fact that the Mo content of plants in Ohaikea Vee was the highest and Pawaena being the second, both Olinda soils of the Latosolic Brown Forest, it would lead to a suggestion that this great soil group is high in available Mo. The soil at Pawaena, however, is a Kapapala loam developed on very shallow phase, while the soil at Ohaikea, the

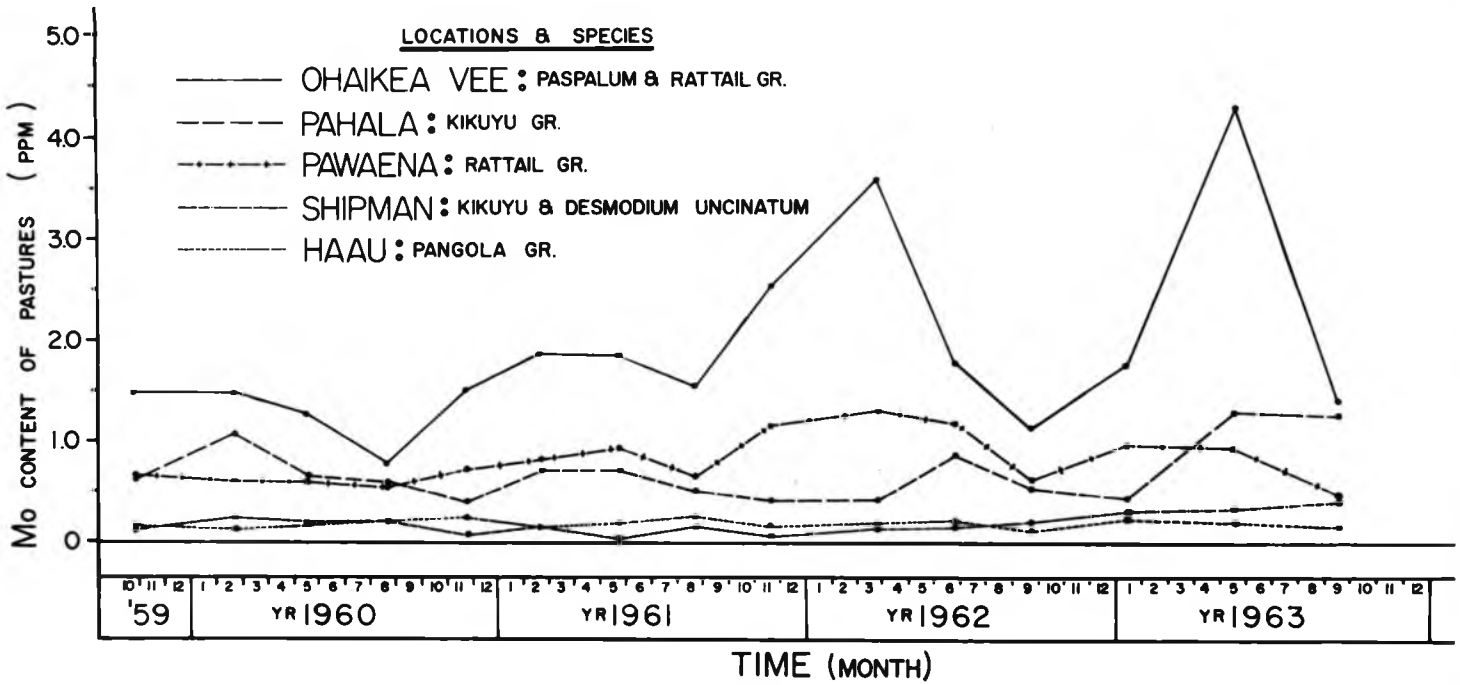

FIGURE 4. A COMPARISON OF SEASONAL VARIATION IN MOLYBDENUM CONTENT OF PASTURE SPECIES GROWN ON FIVE DIFFERENT LOCATIONS.

same soil type, developed on a deeper phase. This deeper phase might possibly give rise to higher available Mo accumulating on the surface than the shallow phase (Pawaena) which is derived from recent volcanic sands occurring between young volcanic material and underlying lava (Cline, et. al. 1955).

The Pahala soil, belonging to the Reddish Prairie great soil group, did not give Mo levels in the plant comparable to that found in Chaikea Vee, but more comparable to that in Pawaena. Based on the fact that Kikuyu grass, which normally requires a low supply of Mo, grew in this soil with the average Mo content of 0.71 ppm. (Appendix I) which is still considered high, the soil is then considered to have a moderate supply of Mo. As mentioned earlier, the Mo content in pastures on Haau and Shipman was very low on the average and this was attributed to the soil factor The Kapoho family of the Humic Latosol (Haau) thus seems to have a low Mo supply and so did the Naalehu family of the Reddish Prairies (Shipman).

It is not possible to make any conclusive statement concerning soil type, soil family, or great soil group in regard to the supply of Mo to the pasture species grown on them because only five soils were involved in this study. Only a large scale study can permit one to do this.

c) Elevation and molybdenum content of pasture species. The elevation seems to have some relationship with the Mo content in herbage species grown on these 5 locations. Chaikea Vee with an elevation of 3,460 ft. and

Pawaena with 2,800 ft. fall in the same high elevation category; these soils gave rise to high Mo content in the herbages. The second category is Shipman and Haau which have nearly the same elevation (1,660 and 1,250 ft., respectively) and have supplied very low Mo level for the herbages. Only Pahala (850 ft.) does not fall into either one of the two categories. It is lowest in elevation and the magnitude of the Mo content of the plants in this location was between the two mentioned categories. A more intensive survey is needed before more detailed conclusions can be drawn. Apparently, there was no relationship between the rainfall and elevation among these 5 locations.

PHASE II

A Preliminary Survey of the Molybdenum and Copper Status of Some Pasture Species and Associated Soils on the Island of Hawaii

a) Possibility of molybdenum toxicity to animals. From the results of Mo analysis in pasture species, there is a strong possibility that Mo toxicity might have occurred in Kukaiau Ranch. The orchard grass in this ranch had a Mo content of 39.0 ppm. which is far beyond the safe limit set by workers in New Zealand (Cunningham, et. al., 1956). It is interesting to note that most of the plant samples taken from this ranch were high in Mo compared to the samples taken from other ranches. Seven plant samples out of 12 collected had Mo higher than 3 ppm. Another area in this survey that gave high plant values of Mo beyond the safe limit for animals is Parker Ranch. Three samples ran higher than 3 ppm. Mo. The lowest Mo content found was from the sample collected from Luke Ranch, the value being 0.08 ppm. This points to a possibility in the opposite direction, Mo deficiency. The range of Mo content for 35 plant samples collected is 0.08 to 39.0 with the average of 4.57 ppm. Ten samples out of 35 had a Mo content higher than 3.0 ppm. and this gave a ratio of 1:3.5 between toxic and non-toxic pasture species. Thus, the occurrence of Mo toxicity to animal due to a high level of Mo present in the pasture plants seems very possible on this island.

b) Copper content of pasture species. Copper content of pasture species collected in this survey, on the other hand, turned out to be very

uniform. The range was 2.6 to 11.8 ppm. with the average of 34 samples being 6.32 ppm. Twelve samples out of these 34 samples had copper contents lower than 5 ppm. This indicates an unfavorable quality of herbage from the standpoint of animal nutrition. Copper content was found to be highest and also lowest in the samples collected from Kukaiau Ranch. As pointed out earlier, Cunningham, et. al. (1956), reported that a Mo content of 1 ppm. in any pasture species is considered adequate for the normal requirement of the plant itself and copper should be around 5 ppm. in order to avoid copper deficiency that might develop in animal. The theoretical ratio of copper to Mo in herbages thus seems to be around 5:1 for optimum animal nutritional requirements. If one were to calculate the ratios from this study, it is found that there were 14 plant samples that gave a Cu/Mo ratio lower than 5. However, the ratio itself can be meaningless and sometimes misleading. The actual contents of either Mo or copper or both have to be taken into consideration. The low ratio with a high copper content tends to be "safer" than the high ratio with only small amounts of copper present as far as copper deficiency in the animal is concerned. The situation is the least favorable when the herbage has a low Cu/Mo ratio and also contains the actually low amounts of copper. There is a strong possibility of this unfavorable situation occurring on Kukaiau Ranch where three samples, orchard grass, mesquite grass, and sweet vernal, contained copper lower than 5 ppm. and also the Cu/Mo ratio were only 0.1, 0.2, 0.1, respectively.

From the results obtained it is indicated further that although several plant samples had a copper content above 5 ppm. the possibility of copper deficiency might still exist. Molybdenum content of these samples was high and might induce copper deficiency in grazing animals. This possibility was indicated for both Kukaiau Ranch and Parker Ranch (Table 3).

c) Total molybdenum and copper content of soils. Considering the data for total Mo in soils. the average of 27 samples was 4.67 ppm. and the values ranged from 1.8 to 8.0 ppm. The highest value was found in the sample collected from Kukaiau Ranch; the lowest from Keahou Ranch. This average Mo value of 4.67 ppm. seems to be quite high when compared to values reported in other areas, especially to the world average value which is roughly 2.0 ppm (Robinson, 1954). However, the higher value on the other islands, especially on Kauai, might be expected (Sherman: personal communication). The total Mo content in the soils from these 27 samples collected seems to be very uniform and this might suggest the uniformity of its distribution in the parent materials on this island.

The total copper content of the soils on the other hand had a very wide range. The deviation from the average value was found to be much greater than that of Mo The average was 83.2 ppm while the range was 10.8 to 185 5 ppm. This wide range of copper content, based on these 27 samples, was found in close agreement with the result of the survey reported by Fujimoto and Sherman (1959) who found the content of the element to range from 16 to 357 ppm.

TABLE 3 SOIL AND PLANT ANALYSIS DATA ON COPPER AND MOLYBDENUM CONTENTS OF SAMPLES COLLECTED FROM 27 LOCATIONS ON THE ISLAND OF HAWAII[1/]

	Soil					Plant		
Location[2/]	Soil pH	Total Mo (ppm.)	Total Cu (ppm.)	Water Extr. Mo (ppm.)	Name of Plant	Mo (ppm.)	Cu (ppm.)	Cu/Mo Ratio
1	6.9	4.6	113.0	.110	Kikuyu gr.	7.3	9.5	1.3
2	6.5	4.9	114.0	.123	"	7.6	9.0	1.2
3	6.6	7.8	59.1	.070	Orchard gr.	39.0*	4.4	0.1
4	5.6	7.9	82.0	.003	Kikuyu gr.	.13	11.8	90.8
					Trefoil	.12	10.8	90.0
5	6.6	7.9	79.8	.140	Kikuyu gr.	17.3	6.2	0.3
6	5.8	8.0	64.0	.042	"	.66	7.2	10.9
					White Clover	.42	3.8	9.1
7	6.3	7.4	24.4	.103	Kikuyu gr.	6.5	8.3	1.2
					Mesquite	23.8	4.2	0.2
8	6.3	3.9	108.4	.057	Sweet Vernal	20.5	2.6	0.1
9	5.8	1.8	47.5	.008	Kikuyu gr.	.50	6.9	13.8
10	6.2	2.6	23.7	.008	Rattail	.15	4.7	31.3
					Trefoil	.39	6.2	
11	5.9	4.8	163.0	.025	Wandering Jew	1.0	5.8	5.8
12	5.6	3.6	83.1	.018	Kikuyu gr.	.29	7.2	24.5
13	5.8	3.3	70.7	.009	"	.36	5.1	14.2
					Trefoil	.18	-	-
14	6.3	2.6	68.0	.031	Kikuyu gr.	.30	4.2	14.0
15	5.9	4.5	59.8	.070	"	1.3	5.6	4.3
16	5.9	2.8	185.5	.015	"	1.1	4.4	4.0
17	6.5	4.5	65.4	.050	"	.46	3.2	7.0
					Clover	.53	6.6	12.5

TABLE 3. SOIL AND PLANT ANALYSIS DATA ON COPPER AND MOLYBDENUM CONTENTS OF SAMPLES COLLECTED FROM 27 LOCATIONS ON THE ISLAND OF HAWAII[1/]

(Continued)

Location[2/]	Soil					Plant		
	Soil pH	Total Mo (ppm.)	Total Cu (ppm.)	Water Extr. Mo (ppm.)	Name of Plant	Mo (ppm.)	Cu (ppm.)	Cu/Mo Ratio
18	6.2	2.9	181.4	.002	Bermuda gr.	.08	4.9	61.3
					Guinea gr.	.36	10.4	28.9
19	6.3	3.8	61.5	.028	Kikuyu gr.	.75	4.8	6.4
20	5.8	5.0	60.5	.006	"	.12	6.2	51.7
21	6.0	4.8	22.2	.012	"	.32	6.3	19.7
22	6.9	2.9	126.7	.036	"	2.1	6.5	3.1
23	6.6	3.0	119.6	.030	"	1.9	4.9	2.6
24	6.8	4.5	76.5	.090	"	7.1	6.3	0.8
					Dutch White	10.2	7.9	0.8
25	6.4	4.3	133.3	.056	Kikuyu gr.	5.7	5.3	0.9
26	5.3	4.6	42.5	.005	"	.25	8.9	35.6
27	5.9	7.3	10.8	.011	"	.94	4.8	5.1

[1/] Each value except pH represents average of two determinations and based on O. D. basis.

[2/] Number corresponding to locations described in Table 1.

*Highest value ever reported in Hawaiian Islands.

in 87 Hawaiian soil samples

d) Total molybdenum in the soils and Mo content in the pasture species. There was no overall relationship between the Mo content of plant species and the total Mo content of the associated soils (Table 3). However, a relationship that was found is that the highest value of Mo in the pasture plants was from the samples collected from Kukaiau Ranch, and also the highest total soil Mo content was obtained from the same location. It is difficult to try to state conclusively at this point that the soil which has high total Mo always tends to give rise to a high value of this element in the plants due to the small number of samples involved in the survey.

The water-extractable Mo did not have any relationship with the total Mo in the soils in this study

This may be the result of the difference in Mo fixation capacity of different soils which depends on the composition of soils themselves such as iron oxides, aluminum status and clay minerals (Jones, 1957). Thus, when fixation is high the total Mo of the soil surface tends to be stabilized and is not subject to leaching, while at the same time the water-soluble Mo may be of a small magnitude. The release of Mo fixed by soil materials also depends to a very great extent on the soil pH. This effect of pH has been known to increase the availability of Mo in the soil solution. On this basis, a soil with a high Mo fixing capacity should release Mo into solution more than with another soil with a low Mo fixing capacity with the same unit increase of pH As stated earlier,

the soils themselves have a wide variation in fixation capacity for Mo and yet the pH of the soil from place to place may not be identical. One would then not possibly get any relationship between the total Mo which is more than 99 percent in the fixed form, or unavailable, and the small amount of Mo in soil solution which is available to plants.

In regard to total copper in the soil and the amount taken up by plants, the same expectations and results were obtained as for Mo There was no correlation between the amount of copper in the plants and the total fraction of the element in the association soils.

e) Relationship between elevation and molybdenum content of pasture species. It appears that elevation exerts some influence on the Mo content of pasture species, the higher the elevation the higher the Mo content in the plants. This relationship can be seen clearly in Kukaiau Ranch where three of the pasture samples collected had very high Mo content. The highest (39.0 ppm.) and third highest (20.5 ppm.) values for all plant samples and the highest (17.3 ppm.) value for Kikuyu grass only were found on this ranch. These three samples were collected from elevations of 7000, 7500, and 5000, respectively, which are among the highest in elevation as compared to the rest of sites. The lower elevations gave lower values of Mo in herbage in general. Although this relationship can not be stated conclusively and confirmably, the trend points to such a possibility Considering the soil formation of this island, the higher elevation soils are much younger than the low elevation soils. According

to Wells' idea (1956) the degree of fixation of Mo by young soils is generally small due to the low content of iron oxide, aluminum oxide and clay minerals, which are largely responsible for absorption or occlusion of Mo. In this case, the upper soil of Kukaiau Ranch, which is considered very young, would likely have a small amount of "active" iron oxides and clay fractions, thus permitting Mo to be free in soil solution. Plants grown in this area, of course, absorbed this element in accordance with the amount present in the soil solution. The soils at the lower elevation, as for example the Waimea Experimental Farm area, are much older and the degree of tropical weathering has presumably proceeded to a greater extent than the Kukaiau Ranch soils. Thus, much of the Mo has been immobilized due to the increase of iron oxides, and the availability of this element in the soil solution is decreased. The plants at the lower elevations generally contained low contents of Mo as can be seen in Table 3.

f) Rainfall influence on molybdenum content of pasture species It appears that rainfall has its influence on the Mo content in pasture species by exerting its influence on the soil pH, which in turn affects the availability of Mo in the soil solution. The other direct effect of rainfall on supplying moisture to the soil and bringing the solubility of nutrients up to the level that plant can absorb easily is not evident according to the results of this study (Table 3).

High rainfall (80-90 inches) presumably contributed to an increased acidity in Mud Lane A and Mud Lane B soils. Kikuyu grass grown on these two soils had Mo content of 0.12 and 0 32 ppm., respectively, as shown in Table 3 Within the same general area but at different sites, Pukalani A and Pukalani B locations have less rainfall annually (35 inches) and the pH of the soils are 6.9 and 6.6, respectively. Kikuyu grass grown on these latter two soils had Mo content of 2.1 and 1 9 ppm. which were much higher than those grown on Mud Lane A and Mud Lane B locations.

This effect of rainfall on soil reaction can be seen further in the Parker Ranch soils, comparing between Puunohu II 1 or Puunohu II 2 and Mud Lane section, and also in Horse Shoe I Ranch (Tables 2 and 3).

g) Relationship between Mo content of Kikuyu grass and water-extractable Mo in soils Wells (1956) believed that young soils tend to have high exchangeable Mo while the mature soils have a very low amount. This exchangeable Mo is gradually fixed during the process of soil formation. Considering the fact that the Island of Hawaii is the youngest island in the Hawaiian chain, soils on this island would tend to give a higher available fraction of Mo than those on other islands. This leads to the expectation that water extraction of Mo in soil would be practical as long as the water-soluble fraction assumes some magnitude. From the results of Barshad (1951) who worked intensively with slightly alkaline California soils, the water-extractable Mo was very high. This high value of water-soluble Mo indicates that the amount of Mo naturally present in

the soil solution would not necessarily be very small as one would imagine. Grigg (1953) in New Zealand found a good correlation between crop response to Mo and oxalic acid-extractable Mo. The use of oxalic acid as the extracting solution at pH 43.3, however, can introduce serious interferences of chromium, titanium, and vanadium in the color development phase unless a separation is made before this step. This involves a complication and is more time-consuming than the water-extractable method. It was, therefore, felt that working with surface soil samples from the Island of Hawaii, the water-extractable method of soil Mo should be tried out to possibly correlate the amount extracted and the amount taken up by the plant.

From the results, it is evident that water-extractable Mo correlated very significantly with the concentration of Mo in Kikuyu grass (Figure 5). This suggests that the amount of Mo extracted would have represented closely the amount of Mo that the plant absorbed from the soil solution.

It has been widely confirmed that increasing the soil pH increases the hydroxyl ion concentration which, in turn, leads to an increase in anions. Conversely, when the pH is lowered, a decrease in the amount of Mo in soil solution can be expected. It is evident that the availability of Mo to plants in a soil is dependent on its pH value. Water has no buffer character and hence when it is used as an extractant, the pH of the soil system will not be disturbed; in turn, the amount of Mo in the soil solution would not be subject to a great change as long as the soil-water ratio is kept to a minimum. This

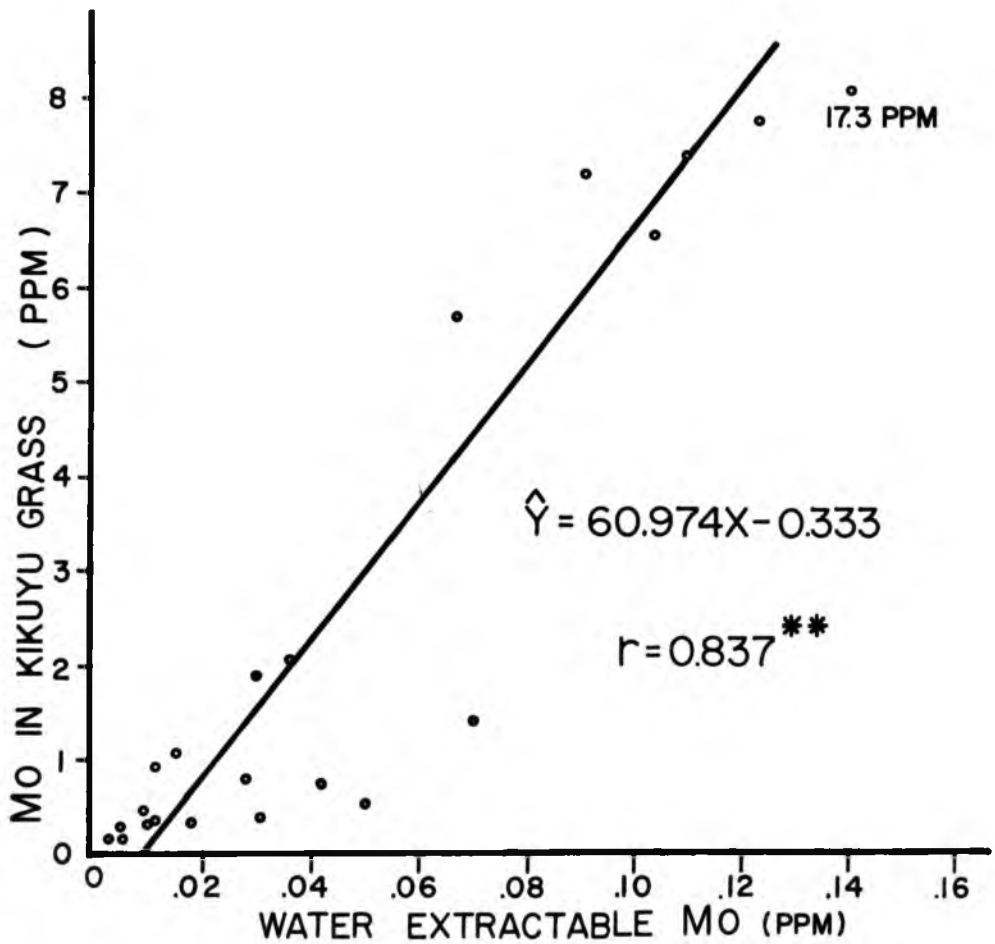

FIGURE 5. CORRELATION BETWEEN WATER-EXTRACTABLE Mo AND Mo CONTENT OF KIKUYU GRASS.

permits one to determine the availability of Mo in soil solution under same conditions as when the plant absorbs the element. As expected, a correlation, significant at the 1 percent level, was obtained between water-extractable Mo in soil and Mo content in Kikuyu grass (see Figure 5). This points out that, as far as Mo is concerned, water extraction can be very practical and a useful tool in assesing the availability of the element in certain soil-plant relationships. However, when working with highly acid soils which fix Mo readily, this method may not be sensitive enough to detect the meager amount of the element in the soil solution.

h) Relationship between soil pH and Mo content of Kikuyu grass. From the results of this correlation study, it was found that Kikuyu grass absorbed Mo from soil according to the amount of Mo in the soil solution. As pointed out earlier, pH exerts a strong influence on the magnitude of the amount of Mo in the soil solution; undoubtedly, a correlation between soil pH and the concentration of Mo in Kikuyu grass can be established. The lower curve in Figure 6 shows that when the pH of the soils increased the content of Mo in Kikuyu grass increased. The Mo content, however, began to rise sharply at approximately pH 6.4 and a cuvilinear relationship resulted. The sharp bending of the curve at pH 6.4 indicates that the availability of Mo in soil solution was drastically increased at this pH. From these 22 soil and Kikuyu grass samples, it can be seen that above pH 6.4 a toxicity due to high accumulation of Mo in the grass is likely to develop anytime and this possibility

will be greater if the soil has a good supply of Mo to start with. When the Mo contents in ppm. values were converted to logarithmic values and a linear regression line was calculated, a highly significant correlation was found between log. Mo and pH (Figure 6).

1) Great soil group and soil family in relation to molybdenum availability. As mentioned earlier, the high accumulation of Mo was found in pasture species growing in Kukaiau Ranch and in Parker Ranch. The Latosolic Brown Forest and Reddish Prairie great soil groups comprise the soils found in these two ranches. This indicates that these two groups have a high available supply of soil Mo. The soils of these two groups have developed from volcanic ash deposits at high elevations and they are similar in many physical and chemical properties. The rainfall is greater in the Latosolic Brown Forest soils than in the Reddish Prairie soils. As a consequence, the base status is lower in the former than in the latter soils (Cline, et. al., 1955). On the basis of water-extractable Mo, which correlated significantly with Kikuyu grass, as a criterion for the Mo status, the soils in these two groups had the highest Mo among all the great soil groups involved in this survey. The soils in Latosolic Brown Forest group as represented largely by the many paddock in Kukaiau Ranch (Table 1) were somewhat higher in Mo status than the Reddish Prairie group. On this ranch the highest availability of Mo was obtained from soils at intermediate elevations, 4500 - 5000 ft. (Tables 2 and 3). There was a considerable variation in Mo availability within the Latosolic Brown Forest

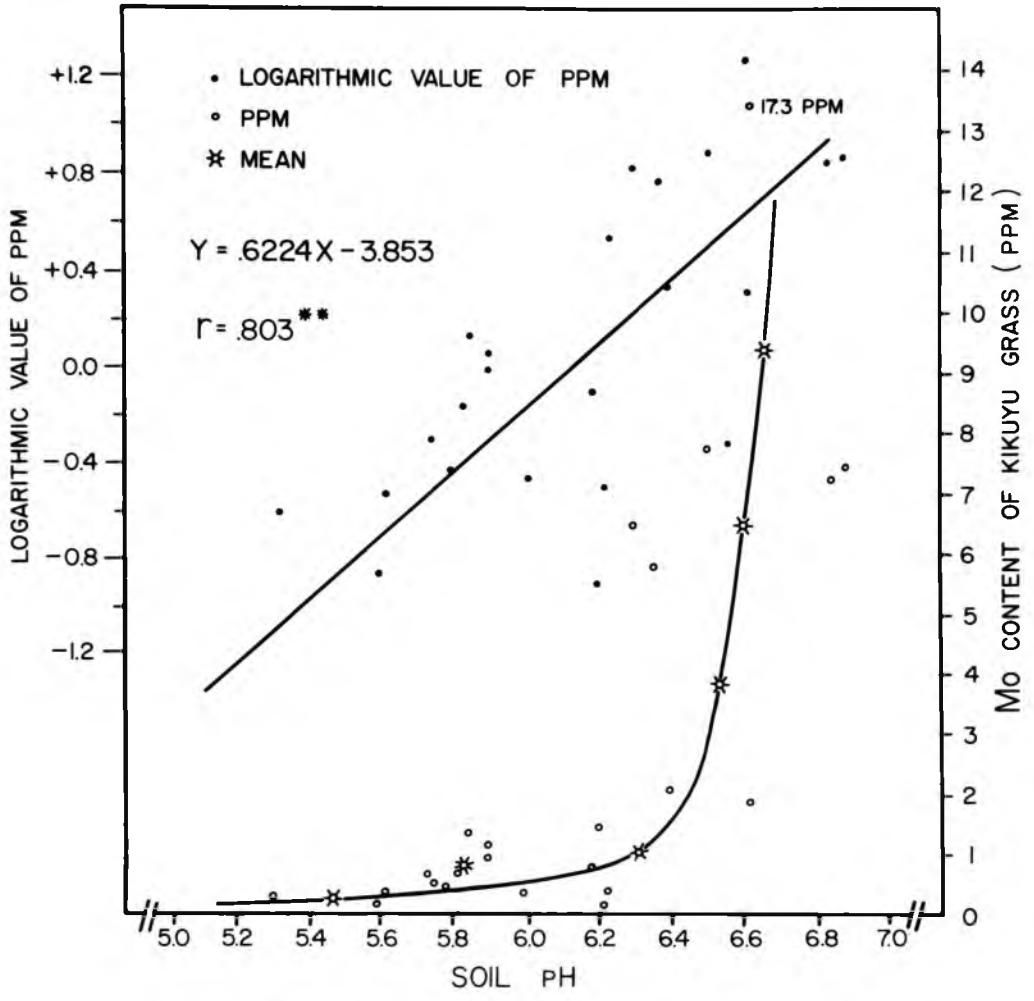

FIGURE 6. RELATIONSHIP BETWEEN SOIL pH AND Mo CONTENT OF KIKUYU GRASS.

group. The higher elevation soils at Kukaiau Ranch under moderate rainfall have pH's approaching neutrality. This gives rise to higher Mo availability in these soils than those developed at lower elevations under more intense rainfall, as in Waimea Experimental Farm and Parker Ranch. Low Humic, Humic, Hydrol Humic Latosols, and Regosols contained much less water-extractable Mo than the average. In spite of this low extractable Mo, the total content of this element was quite high in the Humic Latosol group. This indicates a high fixation capacity of these soils for Mo. The soils in the Hydrol Humic Latosol group, represented by two samples from Horseshoe I Ranch, were somewhat higher in Mo availability than expected. This may be due to the high pH values of these soils, giving rise to higher fraction of Mo in the soil solution. However, the average water-extractable Mo (0.020 ppm.) was still less than the average of Latosolic Brown Forest and Reddish Prairie soils which were 0.051 and 0.034 ppm., respectively. The Low Humic Latosol, as represented by the sample collected from Luke Ranch (Table 1), had a much lower Mo availability than the Hydrol Humic Latosol group and the total Mo content of this soil was also very low. The Regosol, represented by one soil sample collected from Keahou Ranch, had a very low available Mo content as expected. This soil is quite young and shallow and was formed from recent volcanic ash. Apparently the release of Mo has been very small and slow.

Considering soil families within a group, the Hanipoe family in the

Latosolic Brown Forest group showed the highest Mo availability. Water-extractable Mo in these soils ran very high and was the highest of all soils studied (Tables 2 and 3). Orchard grass which was reported to be the highest Mo accumulator found in this survey was grown in this soil family. The pH's of these soils were found to be higher than are usually found for most Latosolic Brown Forest soils. Soils in this family are generally associated with high elevation and moderately low rainfall. Thus, the soil reactions presumably have favored high availability of Mo. The soils were found to be very high in total Mo--the highest in total Mo content among all soils reported in this study. The Maile family, found at a lower elevation than the Hanipoe, had a lower available Mo than the soils in Hanipoe family. The rainfall of this soil family is fairly high and thus greater acidity in this soil can be expected. The Olinda soil, sampled at high elevation and under moderate rainfall, was the lowest in Mo availability of all soils in this group. The pH of the soil sample was 5.75. The order of Mo status of the three soil families in the Latosolic Brown Forest group is Hanipoe $>$ Maile $>$ Olinda.

With regard to total Mo content of soils, soils in the Latosolic Brown Forest group, especially in the Hanipoe family, had the highest contents. As stated earlier, the rainfall in the areas is either moderate or low and the soil reactions are quite favorable for the availability of Mo. The water-extractable Mo was found to be the highest in soils belonging to this group. This indicates

the possibility of a positive relationship between total Mo content in a particular soil with water-extractable Mo when soil pH, and possible sesquioxides are not interfering factors. However, when pH becomes more limiting a much lower Mo availability could be expected. This can be seen in Tables 2 and 3 with the Mud Lane A and Mud Lane B soils, or in Parker Ranch, Kohala section, where total Mo content was high but water-extractable Mo was very low. This is in contrast to the Reddish Prairie soils where the total Mo content was low and yet the water-extractable Mo was higher than the soils at the locations just mentioned previously.

It is reasonable at this point to conclude that elevation, rainfall, and soil reaction are the three important factors that give rise to the difference or variation in Mo availability from one type of soil to another.

PHASE III

Molybdenum Uptake by Plants as Affected by Molybdate, Phosphate, and Sulfate Fertilization

A) The growth of desmodium on four soils

In general, the best growth was obtained in Pukalani and Kahua soils. The average yield per pot was highest in Kahua soil while that in Pukalani soil was second. The lowest average yield was obtained in Mud Lane A soil which had the lowest supply of phosphate and molybdenum (Table 2). Plants grown on Pukalani and Kahua soils were dark green and healthy for all fertilizer treatments. The plants grown on Mud Lane A and B soils appeared unhealthy although receiving P at 500 lb/A. The leaves were yellow-green which may have been due to a nitrogen deficiency, or possibly to a deficiency of micro-nutrient. It is quite obvious that the Rhizobium inoculum applied at the time of planting was not effective on these two soils because nodulation on the roots was very poor, and some of the plants, especially those grown on Mud Lane A soil, had no nodules at all. This suggested very unfavorable conditions in this soil for the growth of rhizobia, and in turn, the apparent lack of nitrogen fixation. Nodulation was good in Pukalani and Kahua soils.

Figure 7 illustrates the growth of Desmodium on the 4 different soils studied. The plants did not receive P or S. Pots on the left were the Mo-controls and those on the right received 2 lb/A Mo. From visual observation

FIGURE 7. THE COMPARATIVE GROWTH OF DESMODIUM TORTUOSUM ON 4 SOILS. (Pots on the left of each pair received no Mo; on the right Mo at 2 lb/A. All pots received no P or S. Soil A = Mud Lane A; Soil B = Mud Lane B; Soil C = Pukalani; Soil D - Kahua -- at 6 weeks).

on the growth of Desmodium the initial fertility status of the 4 soils used in this study increases in the following order. Mud Lane A soil > Mud Lane B soil > Pukalani soil > Kahua soil. Plants grown on Kahua soil showed no visual difference from plants grown on Pukalani soil. However, the overall average yield was slightly higher than that of plants grown on Pukalani soil, and the highest in all soils as mentioned earlier. From Table 2, it is interesting to note that water extractable molybdenum content of the 4 soils lies in the same order as available P, Mud Lane A soil being the lowest while Pukalani soil was the highest. This increasing order fits very well with the degree of molybdenum fixation of the 4 soils. Although the growth of plants on Pukalani soil was not the highest for all soils, the pattern of difference in growth on the 4 soils seems to fit very well with the pattern of phosphate and molybdenum availability which lies in the order: Mud Lane A soil > Mud Lane B soil > Kahua soil > Pukalani soil.

B) Effect of Mo on yield

The effect of Mo applied at a rate of 2 lb/A can be seen in Table 4. The Mo added increased significantly the dry weight yield of Desmodium grown on Mud Lane A soil. The increase was quite consistent for all levels of P in the no sulfate treatment (Figure 8). In the sulfate treatment a significant increase was obtained only between the P_0 and P_{500} level. Although the P_{250} level did not show a significant increase of yield due to Mo application, the trend was still evident. This soil has very high fixation capacity for Mo (Figure 2), and the highest in the 4 soils studied. The initial Mo

FIGURE 8. THE YIELD RESPONSE OF DESMODIUM TORTUOSUM TO PHOSPHATE AND MOLYBDENUM ON MUD LANE A SOIL.

(Pots on the left side of each pair received no Mo; on the right Mo at 2 lb/A phosphate applied in lb P/A. The same trend of response was also observed in Mud Lane B soil at 4 weeks).

status was very low as indicated by the low extractable Mo content (0.006 ppm.) and by the Mo content of kikuyu grass grown on this soil in the area where it was collected (0.12 ppm.).

Response of Desmodium to Mo also was obtained in Mud Lane B soil. Although the difference obtained was not statistically significant, the trend of response is quite evident (Table 4). Although this soil has a somewhat lower degree of Mo fixation as compared to Mud Lane A soil, it still has a very low supply of Mo (Table 2). The response was quite consistent at all levels of P for the no-sulfate treatments but was only slight at the P_{250} level.

Desmodium grown on Pukalani and Kahua soils did not seem to benefit from Mo application. These two soils, however, have higher pH and higher Mo availability (Table 2) when compared to the two soils just discussed. The initial supply of Mo thus had been optimum and was probably above the level normally required by Desmodium. Mo content of the control plants on these two soils was higher than that of the control plants grown on Mud Lane A and B soils (Tables 5 and 6).

C) Effect of phosphorus on yield

Phosphate, similar to Mo, increased the dry-weight yield of Desmodium grown on Mud Lane A and Mud Lane B soils, and did not do so on the Pukalani and Kahua soils. The effect of phosphate on increasing the yield of Desmodium grown on Mud Lane A and Mud Lane B soil was very striking at P_{250}, and a further increase was obtained at P_{500}. Both soils are very deficient in phosphate,

TABLE 4. MEAN WEIGHT OF DRY MATTER YIELD OF DESMODIUM TORTUOSUM GROWN IN GREENHOUSE ON 4 DIFFERENT SOILS (GM./POT)[1/]

SO_4 Added lb/A	P Added lb/A	MUD LANE A Mo Added-lb/A 0	MUD LANE A Mo Added-lb/A 2	MUD LANE B Mo Added-lb/A 0	MUD LANE B Mo Added-lb/A 2	PUKALANI Mo Added-lb/A 0	PUKALANI Mo Added-lb/A 2	KAHUA Mo Added-lb/A 0	KAHUA Mo Added-lb/A 2
0	0	.31	.47	1.03	1.18	3.44	2.98	4.79	3.86
	250	1.44	1.60	1.69	1.74	3.67	3.69	5.02	5.02
	500	1.49	1.80	1.78	1.84	3.59	3.48	5.11	4.80
100	0	.35	.49	1.00	0.87	3.02	3.39	4.85	4.60
	250	1.48	1.55	1.61	1.72	3.30	3.53	4.47	4.05
	500	1.54	1.70	1.68	1.66	3.27	3.42	4.71	4.61
D. 0.05		Mo = 0.15 P = 0.18		Mo = 0.53 P = 0.64		NS		NS	

[1/] Each value represents the average of 4 replicates.

TABLE 5. MOLYBDENUM CONTENT IN PPM. OF DESMODIUM TORTUOSUM GROWN IN GREENHOUSE FOR 6 WEEKS ON 4 DIFFERENT SOILS (O.D. BASIS)

SO_4 Added lb/A	P Added lb/A	MUD LANE A [1] Mo Added-lb/A 0	MUD LANE A [1] Mo Added-lb/A 2	MUD LANE B [1] Mo Added-lb/A 0	MUD LANE B [1] Mo Added-lb/A 2	PUKALANI [2] Mo Added-lb/A 0	PUKALANI [2] Mo Added-lb/A 2	KAHUA [2] Mo Added-lb/A 0	KAHUA [2] Mo Added-lb/A 2
0	0	0.94	16.4	0.21	27.1	1.41	26.3	0.47	2.94
	250	0.18	20.6	0.28	31.9	1.28	35.5	0.41	2.97
	500	0.39	24.3	0.42	30.3	1.34	32.4	0.56	4.54
100	0	0.24	11.6	0.11	11.4	1.54	34.5	0.42	2.58
	250	0.09	18.5	0.20	14.4	1.24	31.0	0.35	3.27
	500	0.36	20.2	0.34	22.5	1.31	30.7	0.52	3.90
D. 0.05						P = NS		P = NS	
						$Mo_{0.01}$ = 13.7		Mo = 1.11	
						SO_4 = NS		SO_4 = NS	

[1] Average of two determinations of composite samples. No statistical inference was made.

[2] Mean of 4 replicates.

TABLE 6. MOLYBDENUM YIELD IN MICRO-GRAM PER POT FOR DESMODIUM TORTUOSUM GROWN FOR 6 WEEKS IN THE GREENHOUSE ON 4 DIFFERENT SOILS (O. D. BASIS)

SO_4 Added lb/A	P Added lb/A	MUD LANE A[1] Mo added-lb/A 0	MUD LANE A[1] Mo added-lb/A 2	MUD LANE B[1] Mo added-lb/A 0	MUD LANE B[1] Mo added-lb/A 2	PUKALANI[2] Mo added-lb/A 0	PUKALANI[2] Mo added-lb/A 2	KAHUA[2] Mo added-lb/A 0	KAHUA[2] Mo added-lb/A 2
0	0	0.29	11.2	0.21	31.9	4.98	100.7	2.16	14.3
	250	0.26	32.96	0.36	55.5	4.70	109.7	2.06	15.0
	500	0.55	41.8	0.62	55.8	4.81	101.8	2.86	21.8
100	0	0.08	6.84	0.11	9.9	4.65	89.2	1.83	11.4
	250	0.13	28.7	0.32	24.8	4.10	129.2	1.56	13.2
	500	2.17	36.4	0.57	37.4	4.31	106.8	2.45	17.9
						P = NS		P = 1.72	
						$Mo_{0.01}$ = 18.3		Mo = 4.60	
						SO_4 = NS		SO_4 = 2.60	

[1] Average of two determinations of composite samples. No statistical inference was made.

[2] Mean of 4 replicates.

and Mud Lane A soil is relatively more deficient than Mud Lane B. A striking increase was obvious in Mud Lane A soil on which the plants receiving no phosphate yielded only 0.31 gm. of total dry weight while the ones receiving 250 lb/A of P weighed 1.44 gm. The response of Desmodium to P on these two soils was quite consistent and significant at every level of Mo and sulfate (Table 4). In Pukalani and Kahua soils, available soil phosphorus seemed to be very high initially (Table 2), and thus further increase in P levels did not show any significant effect on yield. It should also be observed that the yield of Desmodium grown on Kahua soil in the no-sulfate and no-Mo treatments showed a slight tendency to increase in yield with increasing level of P (Table 4). This increase in yield, however, was not significant, and was not consistent at every level of sulfate and Mo.

D) Effect of sulphate on yield

Apparently, the sulfate application of 100 lb/A did not affect the yield of Desmodium significantly. However, a depressing effect on growth was quite evident in Desmodium grown on Mud Lane B soil, Pukalani soil, and Kahua soil. The depression of yield on these soils although not statistically significant, points to the possibility of interaction between sulfate and other soil constituents, possibly phosphorus, molybdenum as other anions. Sulfate might have an effect on pH, but from a measurement of the soil pH after harvesting the Desmodium, only a slight change of $\pm$ 0.2 unit from the original value of each soil was obtained. Thus, the effect of sulfate on pH is considered

very small and insignificant. The explanation of the depression in yield could lie in an interaction between sulfate and phosphate. Sulfate might have depressed the phosphate uptake by Desmodium in the Mud Lane B and Kahua soils, thus limiting plant growth to a certain extent. However, this interaction would not explain the depression of yield in Pukalani soil because initial phosphorus availability in this soil was very high, and an application of sulfate at the rate of 100 lbs/A should not have any appreciable effect on phosphorus uptake by Desmodium grown on this soil. The slight depression of yield for the sulfate treatment in this soil, thus, requires another explanation which is not immediately apparent.

E) Mo content of desmodium

Before presenting a discussion of the results obtained, it should be noted that Mo concentration is rather more meaningful from the standpoint of animal nutrition than is total Mo uptake. However, Mo yield, or the actual amount of Mo taken up per pot also will be presented and used in this discussion when need is indicated.

1) Mud Lane A soil. From the study on Mo fixation it is evident that the greatest capacity to absorb Mo in an unavailable form. The Mo content of Desmodium for control treatments was fairly low, and the average was the lowest obtained for all soils. However, when 2 lb/A was applied, the average Mo content over all P and S levels was strikingly increased. Considering the effect of P on the uptake of Mo there are two trends indicated.

One is that P gave a tremendous response in plant growth and brought about a dilution effect on Mo concentration when the initial supply of Mo was low (Figure 9). However, when 2 lb/A Mo was added, this did not occur. The second trend in effect of P on Mo is that P increased considerably the uptake of Mo as reflected by the Mo content of the plant tissue. This effect of P can be seen clearly from P_{250} to P_{500} when Mo was added, as shown below (mean overall S levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-Mo	0.59	0.14	0.38
Mo	14.00	19.6	22.2

When sulfate was added, there was a decrease in Mo content of the plant at all levels of P. It appeared that this decrease was proportioned for the 3 levels of P. Only at the P_0 level was this decrease quite sharp, but when P was added the difference was smaller. This might be due to the effect of P enhancing the uptake of Mo and thus lessening the depressing effect of S on Mo uptake. Increasing P levels significantly increased the Mo content of the plant regardless of the addition of sulfate. The consistence of S in depressing Mo uptake at every level of P can be seen from the following data (mean overall Mo levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-S	8.7	10.4	12.3
S	5.9	9.30	10.3

FIGURE 9. THE DILUTION EFFECT OF PHOSPHATE ON MOLYBDENUM CONTENT OF DESMODIUM TORTUOSUM GROWN ON MUD LANE A SOIL. (The plants received only phosphate fertilizer at levels indicated in lb/A. Plant on the left contained 0.94 ppm. Mo; on the right 0.18 ppm.).

Considering simultaneously the effect of S and Mo applications on the Mo content of Desmodium grown on this soil, the results presented below clearly indicate the depressing effect of S on Mo uptake and the significant increase (more than 40 fold) in Mo content of plants receiving 2 lb/A Mo (mean overall levels, Table 5, ppm.).

	No S	S
No-Mo	0.50	0.23
Mo	20.4	16.8

2) Mud Lane B soil. The Mo content of the control plants was fairly low and this value was significantly increased when Mo was added. The overall average Mo content in the plants grown on this soil was slightly higher than that on Mud Lane A soil which is in full agreement with the degree of Mo fixation for the two soils. Mud Lane A soil has the highest fixation capacity for Mo (Figure 1). The availability of Mo in Mud Lane B soil would therefore be somewhat greater. Here again, the effect of P on enhancing the uptake of Mo is clearly evident, and the trend was the same for both the Mo and no-Mo treatments. P did not cause a dilution effect on Mo as was the case with Mud Lane A soil. Mud Lane B soil has a somewhat higher P initial level (Table 2), and hence, the response of Desmodium to P fertilization was not as marked. Thus, when no Mo was added, the general trend was that Mo content in the plant increased with increasing levels of P. This trend was marked and was found in both the no-Mo and Mo treatments

as shown by the following data (mean overall S levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-Mo	0.16	0.24	0.38
Mo	19.3	23.2	26.4

The increased uptake of Mo resulting from addition of P apparently had not reached a maximum, and it is possible that further increases could be expected at rates of P beyond 500 lb/A.

When sulfate was applied to this soil the depressing effect on Mo uptake was found to be very marked. Sulfate application reduced the Mo content of the plant at every level of P, and the reduction was very sharp when no P was added to the soil. However when 500 lb/A of P was added the decrease in Mo content due to sulfate depression was much less. This interaction in both Mud Lane A soil and this soil can be explained as resulting from higher levels of P decreasing the depressing effect of S on Mo uptake. The effects of sulfate and P on Mo content of Desmodium grown in this soil can be seen in the data below (mean overall Mo levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-S	13.7	16.1	15.4
S	5.8	7.3	11.4

When the effects of S and Mo are considered together it is evident that S depressed Mo uptake by the plants at both levels of Mo. Mo application

brought a significant increase in Mo content of the plants for both the no-sulfate and sulfate treatments. However, the level of Mo in the plant was decreased by almost half due to the addition of sulfate to the Mo-treatment as shown by the data below (mean overall P levels, Table 5, ppm.).

	No S	S
No-Mo	0.30	0.22
Mo	29.8	16.1

3) Pukalani soil. This soil has the highest pH (6.9), and in the fixation study (Figure 1), it exhibited the lowest fixation capacity for Mo. As would be expected, Mo tended to be more readily available to the plant than in the other soils. The high Mo content found in plants grown on this soil clearly indicates this greater availability. The average concentration for plants grown on this soil was 16.54 ppm. It is to be noted that application of 2 lb/A Mo to this soil gave a tremendous increase in Mo content in the plants over the control, and this increase was highly significant at the 1 percent level, (Tables 5 and 6). This could certainly be attributed to the high pH, and possibly to the high phosphate level of the soil. Because of the high phosphate level it might be expected phosphate would not enhance the uptake of Mo. From the data obtained (Table 5), this was found to be true. No significant increase was obtained. In fact, for the no-Mo treatment, the highest level of Mo was found in the plants receiving no additional phosphate. The data are summarized below (mean overall S levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-Mo	1.5	1.3	1.3
Mo	30.4	33.3	31.6

The increase in Mo concentration due to Mo application was about 25 fold. The increase and decrease of this element due to phosphate application did not assume any significant magnitude.

Regarding the sulfate effect on the content of this element, it was found that there was no significant difference in either a positive or negative direction. The plant seemed to take up Mo uniformly regardless of the addition of sulfate. There was a trend, however, in that sulfate slightly depressed Mo uptake in the P_{250} and P_{500} treatments. It should be noted here that the P_0 level sulfate seemed to increase the Mo content and that this increase approached significance. There is no immediate explanation for this effect. The results are summarized below (mean overall Mo levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-S	13.9	18.4	16.9
S	18.0	16.1	16.0

Considering the effect of the sulfate-Mo interaction on the Mo content of Desmodium grown on this soil, Mo application increased the Mo content in the plant significantly over the control at both levels of sulfate. Sulfate, as stated earlier, did not give any depression effect on Mo content as indicated by the data below (mean overall P levels, Table 5, ppm.).

	No S	S
No-Mo	1.3	1.4
Mo	31.4	32.1

4) Kahua soil. In general, the Mo content of the plants in the control treatment was lower than that found in Pukalani soil and a little higher than the averages of both the Mud Lane A and Mud Lane B soils. The application of 2 lb/A Mo resulted in a significant increase in Mo content of the plants. However, it was found that the average content of Mo in the plants receiving 2 lb/A of Mo was only 3.02 ppm. Yet, from the fixation study (Figure 1), this soil did not have as high a fixation capacity for Mo as did the Mud Lane A and Mud Lane B soils. Plants growing on the latter soils had an average Mo content of about 16 ppm. when Mo was applied. Hence, the low Mo content of the plants grown on Kahua soil cannot be explained on the basis of the Mo fixation capacity. The question remains open as to why plants grown on this soil did not take up Mo in the same proportion as those grown on other soils that received the same rate of application of Mo and yet had a higher capacity for Mo fixation. From the yield data, plants grown on this soil produced the highest yield and were very healthy. It is unlikely, however, that this high yield resulted in a dilution effect since it did not differ greatly from that obtained on the Pukalani soil.

Considering the effect of phosphate on the Mo content of Desmodium grown on this soil, it was found that P did not significantly change the amount

of Mo taken up by the plants when no Mo was added. This might be attributed to the low initial supply of Mo which would limit the amount of Mo available to the plants, even when the phosphate level was increased. However, when Mo was added, an increase in Mo content was obtained (Tables 5 and 6) with increasing levels of phosphate as shown by the data below (mean overall S levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-Mo	0.45	0.38	0.54
Mo	2.8	3.1	4.1

This increase due to Mo fertilization was not statistically significant due to wide variations between replicates within a treatment. However, the trend does show that the increase in Mo content was mainly due to the effect of increasing levels of P in the soil. This trend was also observed in the control treatment in which application of P at 500 lb/A gave a slight increase in Mo content over the 250 lb rate.

When sulfate was added to the soil, a slight decrease in Mo content was observed at the P_0 and P_{500} levels. This difference was not statistically significant, however. (Mean overall Mo levels, Table 5, ppm.).

	P_0	P_{250}	P_{500}
No-S	1.7	1.7	2.6
S	1.5	1.8	2.2

When the effects of Mo and sulfate on Mo content of the plant are considered together over all phosphorus levels, the data show that the Mo content of Desmodium grown on this soil was decreased by application of sulfate. This effect is indicated by the following data (mean overall P levels, Table 5, ppm.).

	No S	S
No-Mo	0.48	0.43
Mo	3.5	3.3

This difference was small and not significant. However, it should be noted that application of Mo at 2 lb/A increased significantly the content of this element in both the no-sulfate and sulfate treatments.

P content of Desmodium

In general, when comparing the four soils, it was evident that Pukalani soil had a high initial supply of available P. The plants took up more phosphate than from other soils, and no response to additional P was obtained. The average P content of plants grown on this soil was 0.214 percent, while the average P content of plants grown on the other soils decreased in the order Kahua soil, Mud Lane B soil, and Mud Lane A soil. The lowest average P content in plants grown on Mud Lane A soil was expected from the very low available P content of this soil (see Table 2). Increasing levels of P application in general, brought a significant increase in P content of the plants grown on

Mud Lane A soil, Mud Lane B soil, and Kahua soil while plants grown on Pukalani soil did not show any significant change in P content after P application. Phosphorus concentration in plants grown on Mud Lane A soil increased greatly between the 0 and 500 lb/A rates. However, P concentration in plants grown on this soil still did not approach the high level (0.26 percent) obtained on Pukalani soil (Table 7). This points to a suggestion that phosphate fixation capacity of Mud Lane A soil is much greater than that of Pukalani soil.

This difference in phosphate fixation capacity is probably related to pH of the soil (see Table 2). Mud Lane A soil, having the lowest pH, may have a high fixation capacity as reflected by the extractable P content which was initially only 4 ppm. Extractable P content of the Pukalani soil (pH 6.9) was 660 ppm.

In both Mud Lane A and Mud Lane B soil, P application from 0 to 500 lb/A increased P content in the plant at every level of Mo and S. In Mud Lane A soil, the greatest increase was between 0 and 250 lb/A, with a slight additional increase at the 500 lb rate. In Mud Lane B soil the increase seemed to maintain a linear relationship, and at P_{500} the maximum was still not reached. The behavior of these two soils was similar as regards changes in plant P due to application of P which might suggest similar properties of the two soils. However, Mud Lane B soil, which has a higher extractable P content (around 12 ppm. - 0.02 N H_2SO_4), gave rise to a higher P content in the plant at P_0

TABLE 7. PHOSPHORUS CONTENT IN PERCENT OF DESMODIUM TORTUOSUM GROWN FOR 6 WEEKS IN GREENHOUSE ON 4 DIFFERENT SOILS (O.D. BASIS)

SO_4 Added lb/A	P Added lb/A	MUD LANE A[1/] Mo Added-lb/A 0	MUD LANE A[1/] Mo Added-lb/A 2	MUD LANE B[1/] Mo Added-lb/A 0	MUD LANE B[1/] Mo Added-lb/A 2	PUKALANI[2/] Mo Added-lb/A 0	PUKALANI[2/] Mo Added-lb/A 2	KAHUA[2/] Mo Added-lb/A 0	KAHUA[2/] Mo Added-lb/A 2
0	0	.069	.071	.104	.110	.201	.220	.168	.172
	250	.147	.152	.136	.148	.216	.176	.200	.169
	500	.182	.167	.182	.170	.189	.227	.231	.240
100	0	.054	.048	.097	.076	.190	.224	.143	.175
	250	.145	.150	.143	.138	.240	.257	.163	.196
	500	.170	.183	.178	.163	.218	.208	.224	.217
D. 0.05						NS		P = 0.035	

[1/] Average of two determinations of composite samples. No statistical inference was made.

[2/] Mean of 4 replicates.

than did Mud Lane A soil.

In Kahua soil, although the initial level of P was already high, an increase in P uptake was still evident when P was added to the soil. A significant increase was obtained in both Mo and S treatments. When Mo and S were not added the increase was significant only when comparing the P_0 and P_{500} levels. The P_{250} level did not give any significant change from the P_0. This might be due to the fact that this soil has a moderately high available P content (148 ppm.); thus a small addition of P to the soil may not alter the rate of uptake to a measurable extent. In the treatment with sulfate the average P content of the plant was somewhat lower than the treatment without sulfate. This might be attributed to sulfate competing with phosphate for uptake, thus lessening the P content in the plant. From Table 6, it is evident that sulfate depressed the uptake of phosphate in Mud Lane B and Kahua soils. This interaction will be more fully discussed with the P-S-Mo interaction.

G) Sulfur Content of Desmodium.

In the four soils studied application of sulfate did not significantly increase the sulfur content of the plants except on Kahua soil (Table 8). On this soil significant differences were obtained where Mo had been applied, but not on the treatments not receiving Mo. It may be pointed out here that increasing P tended to exert a depressing effect on the sulfur content of plants grown on Mud Lane B soil. However, this decrease due to P was not significant.

TABLE 8. SULFUR CONTENT IN PERCENT OF DESMODIUM TORTUOSUM GROWN FOR 6 WEEKS IN GREENHOUSE ON 4 DIFFERENT SOILS (O. D. BASIS)

SO_4 Added	P Added	MUD LANE A[1]		MUD LANE B[1]		PUKALANI[2]		KAHUA[2]	
		Mo Added-lb/A		Mo Added-lb/A		Mo Added-lb/A		Mo Added-lb/A	
lb/A	lb/A	0	2	0	2	0	2	0	2
0	0	.363	.346	.318	.367	.215	.237	.292	.237
	250	.317	.312	.250	.285	.168	.197	.246	.333
	500	.385	.360	.321	.295	.282	.256	.354	.216
100	0	.467	.485	.630	.407	.260	.237	.314	.403
	250	.436	.496	.575	.398	.307	.221	.287	.316
	500	.303	.580	.499	.432	.251	.348	.390	.387
D. 0.05						NS		S =	.128

[1] Average of two determinations of composite samples. No statistical inference was made.

[2] Mean of 4 replicates.

As mentioned earlier the initial availability of phosphate in Pukalani soil was very high and thus gave rise to the highest uptake of P by Desmodium. In contrast, the average sulfur content of plants grown on this soil was found to be the lowest compared to other soils. Increasing levels of P did not decrease the sulfur content any further. The highest sulfur content was found in plants grown on Mud Lane A soil which had the lowest supply of phosphorus. The sulfur content of Desmodium on these two soils, Mud Lane A and Pukalani, thus appears to be inversely related to the phosphate level.

Molybdenum application did not have any effect on sulfur content of the plant although sulfate application resulted in a significant depression of Mo uptake, as discussed earlier.

H) Phosphorus X Sulfur X Molybdenum interaction.

This interaction occurred to some extent in Soil A and to a greater extent in Soil B when 100 lb/A sulfate was applied. The content of P was decreased when sulfate was added. This interaction between sulfate and phosphate reflected to a great extent Mo absorption by the plant. The level of Mo decreased considerably when sulfate was added. In the opposite direction, P tended to exert a more competitive effect on sulfate in Soil B, (Table 8) for in the sulfate treatment the sulfate content in the plant decreased appreciably with increasing levels of P. Here again, molybdenum uptake increased significantly when the level of P increased. This interaction was not distinct

in Kahua soil and was not observed at all in Pukalani soil. There was no explanation as to why the overall average sulfur content of the plants grown on Pukalani soil was lowest compared to other soils since there was no indication of sulfate being depressed by increasing P levels. However, it should be pointed out that Pukalani soil had the highest initial supply of P (Table 2), and the phosphate content of the plants was also high. This initially high availability of phosphorus probably decreased sulfate uptake and enhanced Mo uptake to such an extent that additional phosphorus could not exert any further effect.

As mentioned above this P-S-Mo interaction was not as pronounced on Kahua soil. However, when all three fertilizers were applied together the sulfur content of the plants tended to decrease (Table 7) with increasing rates of added P.

A general interpretation of the findings may be summarized as follows:

1) Increasing levels of P increased the competition between P and S resulting in decreased uptake of S (anion competition for absorption sites on the root surface).

2) When sulfur absorption was decreased by increasing levels of P there was a simultaneous increase in Mo uptake by the plants.

3) Sulfate anions, when present in the soil solution at concentrations used in this study, will compete directly with molybdate anions for absorption sites on the plant root. The resulting depression in Mo uptake will be more

pronounced when P status in the soil is low. Increasing rates of P tend to lessen the antagonistic effects of applied sulfate.

PHASE IV

The Availability of Molybdenum in a Simplified Soil System as Influenced by pH, Phosphate and Sulfate Concentrations

As described in the Materials and Methods Section, Akaka soil consists largely of active amorphous iron and aluminum oxides which are responsible for the high phosphate fixation capacity of this soil (Fox et. al. 1962). From the results of this experiment, it was found that Akaka soil fixed most of the Mo added resulting in the least amount of Mo remaining in the soil solution. Thus, fixation of phosphate and molybdate might involve the same mechanism and fixing agents in a given soil. Molybdate is believed to be fixed at the surfaces of soil colloids; hydrous iron oxide, hydrous alumina and clay colloids such as halloysite, kaolinite, and nontronite (Jones, 1957). This fixed molybdate can be made available by addition of hydroxyl ions into the soil solution as shown clearly in this experiment (Figure 10). Between pH 3 and 5.5 the amount of Mo remaining in the soil solution was very small. The curve, however, rose sharply at around pH 7 and up to pH 8.5 and then tended to level off. This indicates the region of maximum exchange between molybdate and hydroxoyl anions, and it also indicates that the maximum capacity of hydroxoyl anions to effectively release molybdate anions from the soil solids was nearly reached. As stressed earlier, the soil had a very large amount of hydrous iron oxides and alumina present, and the largest amount when compared (O. D. basis) to Naiwa soil

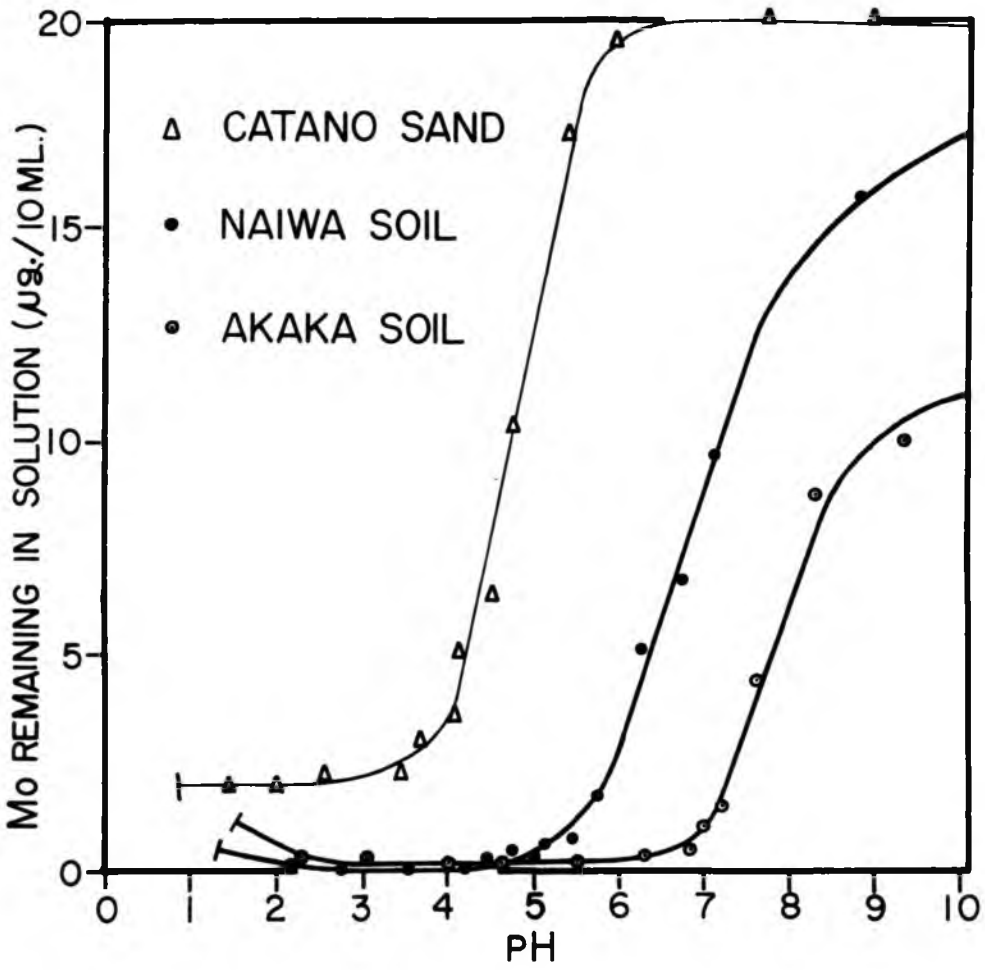

FIGURE 10. RELATIONSHIP BETWEEN SOIL pH AND Mo AVAILABILITY IN SOIL SOLUTION FOR 3 SOILS STUDIED.

and Catano sand which were also used for this study. It exhibited the strongest fixation capacity for Mo and has the widest pH range (pH 3 to pH 7) over which maximum amounts of Mo will be adsorbed.

Naiwa soil, which contains a large amount of iron oxides and titanium, did not turn out to be the strongest fixer for Mo. This might be due to the fact that the soil sample used had been air dried before the experiment started. Thus the capacity of the iron oxides and clay colloids in this soil to fix Mo was lessened to a certain extent. This soil fixed less Mo than Akaka soil, and has a narrower range of pH over which maximum adsorption occurred. However, it is very interesting to note that this soil had a greater fixation capacity than Akaka soil from pH 2 to pH 4, for no Mo was detected remaining in the solution over this pH range. The sharp bend in the curve for this soil (Figure 10) occurred at about pH 5.3 which is about 2 units lower than for Akaka soil. Fixation decreased sharply up to about pH 9 and the curve then started to level off. This maximum capacity of hydroxoyl anions to increase the availability of Mo in the soil solution is considered somewhat greater than that found in Akaka soil.

The Catano sand was found to be a weak fixer of Mo as had been expected. The pH range where maximum adsorption occurred was narrow, probably the narrowest, and yet the amount of Mo remaining in the soil solution over this pH range was found higher than in the first two soils. The point emphasized here is that adsorption of Mo added at a concentration of 200 g. per 100 ml. at this pH range reached saturation before the entire amount of

Mo was removed from the solution. The bending of the curve began at a pH of about 3.5 and proceeded up until all the Mo adsorbed was recovered at pH 6. Beyond this pH the curve then became horizontal. It should be pointed out that in obtaining the low pH values for this soil most of the coral sand was dissolved and placed in solution. The alluvial soil materials remaining were responsible for the fixation of molybdenum.

All the curves had certain features in common, each displaying a sigmoidal shape. There were fairly wide ranges of pH over which the three soils adsorbed maximum amounts of molybdate. The order in which they adsorbed molybdate is: Akaka $>$ Naiwa $>$ Catano sand. The degree of Mo adsorption of each soil, however, will depend on the initial concentration of Mo added (Jones, 1957), the volume of solution used, and also the amount of soil in the simplified system. This will not change the sigmoid characteristics of the curves but may change their positions slightly by shifting them either to the left or to the right on the pH scale. Maximum adsorption of Mo by the two soils and sand was found to occur in the moderately acid range. Inhibited adsorption by these soils found occurring at higher pH values fits well with the experiments of Barshad (1951) who reported that adsorption of molybdate was progressively inhibited when the pH of the system was raised over the range 5.0 to 9.0. The results obtained from this study, concerning the pattern and general features of the "Mo adsorption curve" were found to agree with those of Jones (1957). He stated that the decrease in Mo remaining

in soil solution should be attributed greatly to adsorption by sesquioxides and other clay colloids rather than to precipitation of molybdate by iron and aluminum.

The effect of phosphate concentration on the availability of Mo in the soil solution was found to be very positive in increasing the amount of Mo remaining in solution. Increasing the concentration of phosphate resulted in an increase in the amount of Mo which could be expressed exponentially on the graph (Figure 11, 12, 13). The effect of sulfate was in the same direction, the amount of Mo remaining in the soil solution being increased but to a very small degree when compared with the effect of phosphate. This increase in Mo remaining in the soil solution points strongly to the presence of an anion exchange between Mo and phosphate or sulfate.

As mentioned before, in order to compare the effects of phosphate and sulfate on Mo availability, the three soil materials were used in equal amounts on an oven dry basis, and the pH was adjusted to 5.9 ± 0.05 for every soil and treatment. Figure 11 reveals the exponential nature of the curve of Mo availability which rose gradually as the concentration of phosphate increased. Availability increased more and more slowly until at 1500 ppm. phosphate saturation occurred. Beyond this phosphate concentration, the curve tended to be a straight horizontal line.

Sulfate reached its maximum capacity to replace molybdate much sooner than phosphate. The maximum of the curve occurred at 300 ppm.

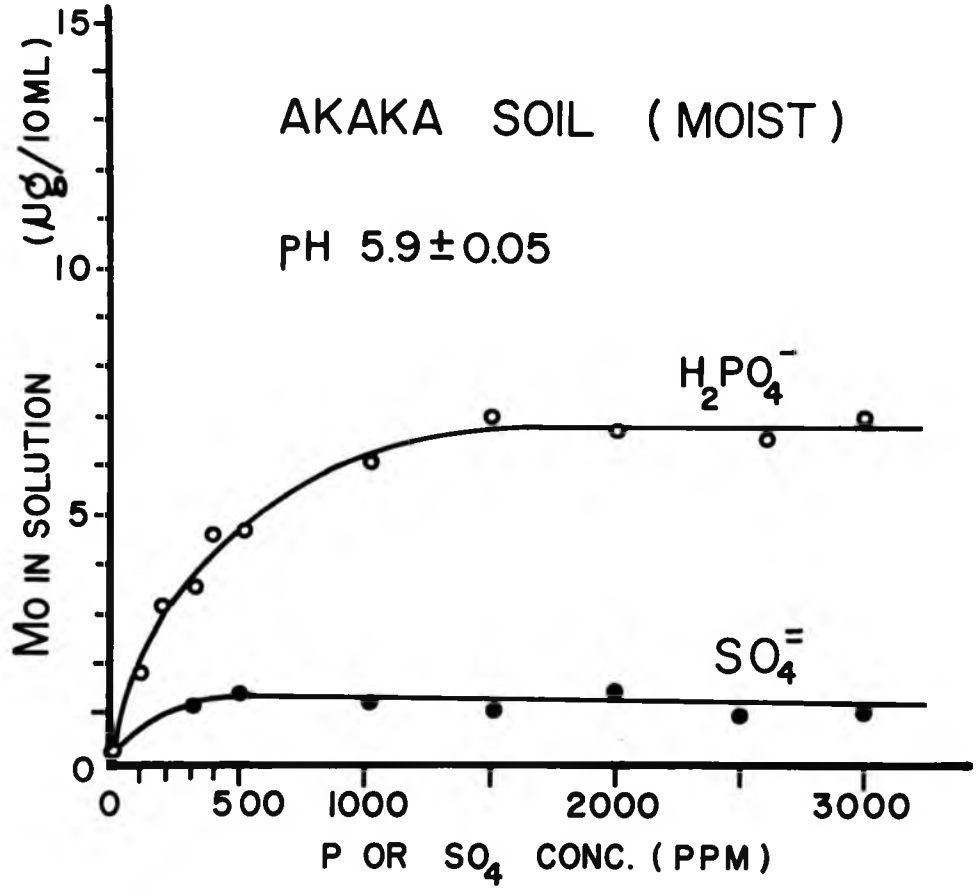

FIGURE 11. THE INFLUENCE OF PHOSPHATE AND SULFATE CONCENTRATIONS ON Mo AVAILABILITY IN SOIL SOLUTION (AKAKA: MOIST).

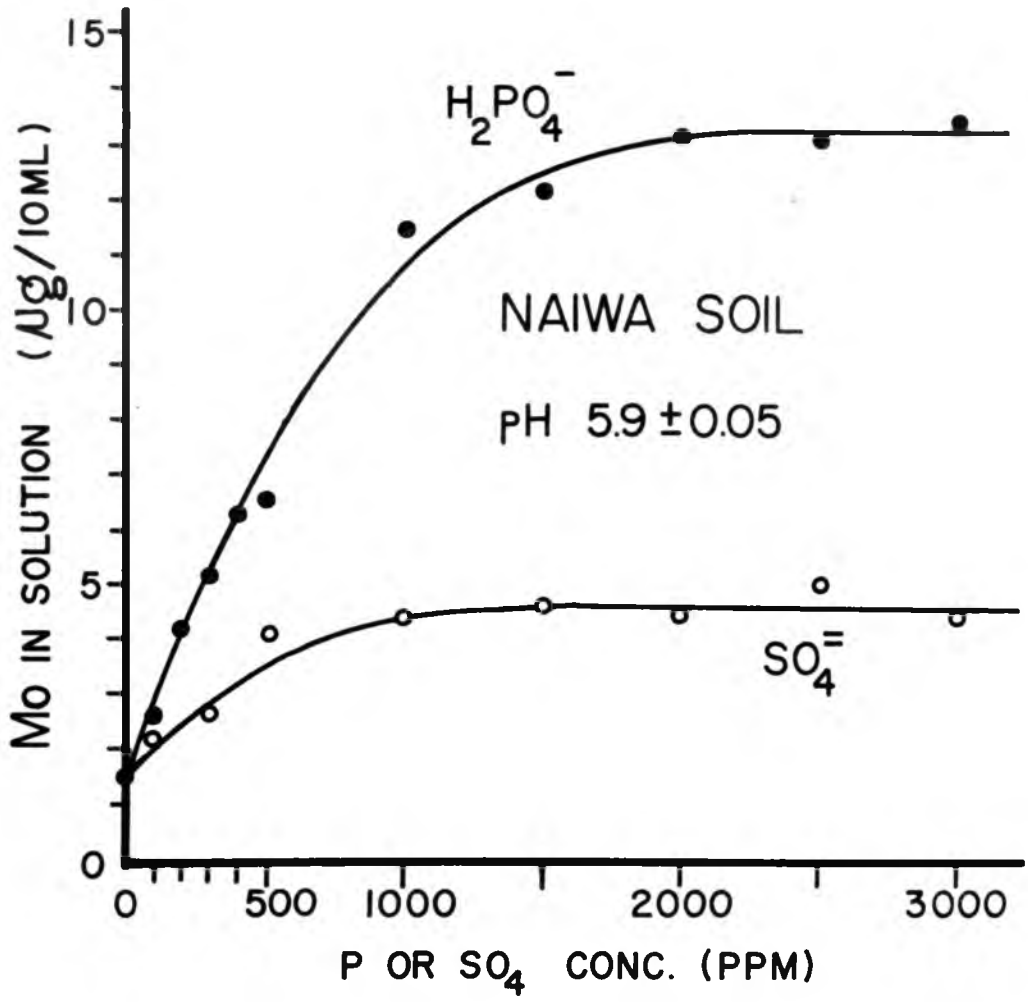

FIGURE 12. THE INFLUENCE OF PHOSPHATE AND SULFATE CONCENTRATION ON Mo AVAILABILITY IN SOIL SOLUTION (NAIWA).

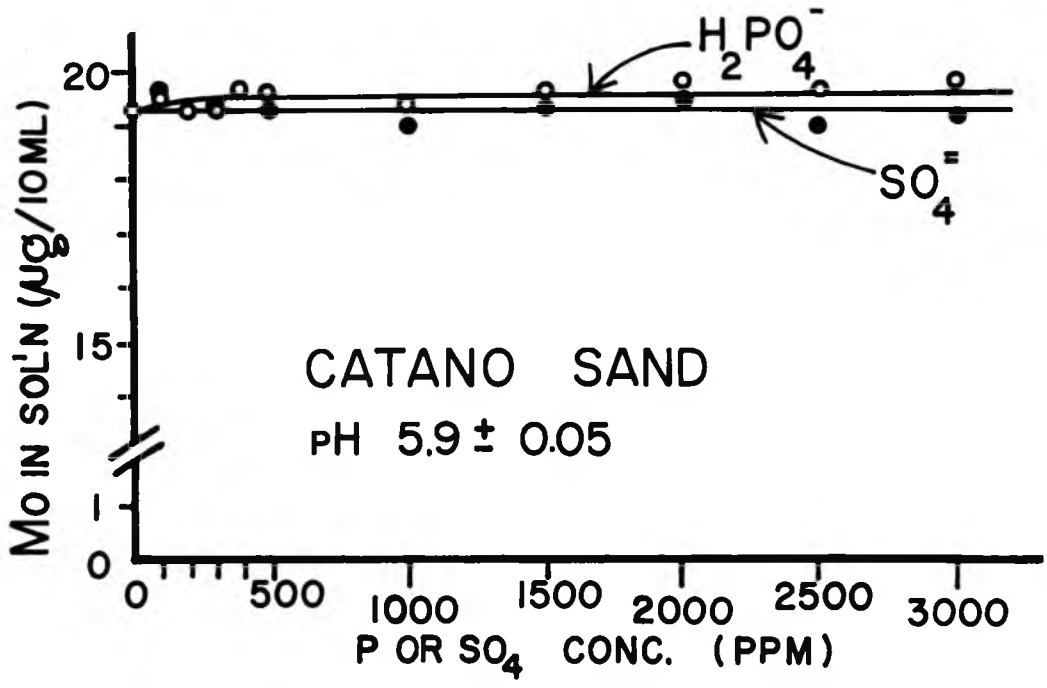

FIGURE 13. THE INFLUENCE OF PHOSPHATE AND SULFATE CONCENTRATIONS ON Mo AVAILABILITY IN SOIL SOLUTION (CATANO SAND).

SO_4 (Figure 11). This difference could possibly be attributed to a higher affinity of clay colloids for phosphate than sulfate, thus allowing phosphate to exchange with molybdate more extensively than sulfate. It was noted also that Akaka soil had the greatest adsorption capacity for Mo and hence allowed a lower maximum amount of Mo to stay in the solution phase of the system (7.0 μg./ 10 ml.). This increase of Mo in the solution, however, is calculated roughly to be about 35 fold over the initial Mo level when phosphate was not added. This increase as a result of phosphate addition was somewhat less than the increase due to the effect of hydroxoyl anions as discussed previously.

Naiwa soil ranked second for Mo adsorption. The level of Mo in the solution at the maximum effect of phosphate was found greater than in Akaka soil (around 13.5 μg./10 ml.). The curve tended to rise as a straight line as the concentration of phosphate increased from zero (Figure 12). The effect of phosphate however reached a maximum at 2,000 ppm. resulting in a horizontal curve beyond this level. The effect of sulfate was much lower in magnitude than phosphate and yielded a curve very similar but proportionately lower than that due to phosphate effects. The saturation effect of sulfate on releasing Mo into solution in this soil occurred around 1,000 ppm. which was greater than the concentration that gave the saturation in Akaka soil.

Catano sand, while being third in degree of adsorption of Mo, did not give a clear effect of phosphate or sulfate on replacing molybdate anions adsorbed

at the selected pH (5.9). A slight exponential effect was obtained for phosphate, but sulfate resulted only in a straight line parallel to the horizontal axis (Figure 13). This is a direct result of the nature of the sand itself which fixes only slight amounts of Mo at pH 5.9. The amount of Mo in solution was already high at the beginning of the test. Adsorption of Mo was thus very small in magnitude at this pH and any release due to replacement by phosphate was hardly measurable. Hopefully, the effect of phosphate and sulfate concentrations on the availability of Mo in the solution would have been the same as found in Akaka soil and Naiwa soil. Similar results might be obtained if the selected pH were 4.0 (according to the curve obtained with various pH values Figure 10). At this pH the amount of Mo remaining in solution would be around 3.5 µg./10 ml. from which 6.5 µg. of Mo would be adsorbed and replaced by the phosphate and sulfate anions.

SUMMARY AND CONCLUSION

The thesis problem was divided into four separate studies, each closely related to the others. The first phase involved a study of the level of Mo in pasture herbage grown at five different locations as influenced by seasonal variation. Rainfall was found to exert a dominant effect on the Mo content of pasture species grown at two locations, Ohaikea Vee and Pawaena. It had only a small influence on the third location, Pahala; while no influence was noted at Haau and Shipman paddocks. The rise and fall of Mo level in pastures on the first two locations followed closely the pattern of rainfall distribution. This is probably due to the fact that rainfall increases and decreases the level of soil moisture and thus controls the availability of Mo in the soil. At Haau and Shipman, the Mo content in the plant was very low and rainfall was well distributed throughout the year, thus supporting a continuously high moisture level in the soil and lessening to a great extent the degree to which Mo availability was dependent on rainfall. Data on elevation, great soil group, and soil family also showed some evidence of relationship with Mo level in the pastures. The Latosolic Brown Forest soils tended to give a higher Mo supply than the Humic latosol and Reddish Prairie soils as indicated by plant uptake of this element.

The second phase of the thesis deals with a survey of the Mo and Cu levels in some pasture species and associated soils from the Island of Hawaii.

There was a strong indication that Mo toxicity might have developed in some areas of Kukaiau Ranch. The highest value obtained was 39.0 ppm., Mo which was found in Orchard grass collected at an elevation of 7,000 feet from this ranch. The data obtained indicates that soils at the high elevations with low to moderate rainfall tended to give rise to a high level of Mo in the herbage. There was also an indication of copper deficiency in some areas and, from the standpoint of animal nutrition this would be very undesirable, when the Mo content is high. The average total soil Mo from 27 samples was 4.67 ppm., and the deviation from the average was quite small, thus indicating a uniform distribution of this element in these soils. On the other hand, total copper content in the soils showed a wide deviation from the average value which was 83.2 ppm. This wide variation in copper content in the soil was in close agreement with the results of Fujimoto and Sherman (1959). There was no correlation between the amounts of the two elements taken up by plants and the total soil content using Kikuyu grass as indicator plant. However, water extractable Mo was significantly correlated with Mo content in Kikuyu grass. This emphasized strongly that the water extraction method for available molybdenum is more meaningful and may be superior to the method used by Grigg (1953) in New Zealand. His method is more complicated and is also subject to interference from the heavy metals, V, Cr, and Ti. However, it appears that the water extraction method can be applied successfully only to young soils that tend to contain higher levels of soluble Mo due to their

relatively high pH and low sesquioxide content. Correlation between soil pH and Mo content of Kikuyu grass was also found to be significant in this study. A straight line regression was obtained when the logarithym of the Mo concentration was plotted against soil pH.

The third phase of this work was an investigation of Mo uptake by a test plant, Desmodium tortuosum, as influenced by phosphate and sulfate and also the application of molybdenum. A slight response of Desmodium was obtained from Mo addition to the Mud Lane A and Mud Lane B soils. Application of phosphate resulted in a tremendous growth response on these two soils but not on the Pukalani and Kahua soils. Sulfate application did not significantly increase or decrease plant yield, but there was a trend toward sulfate depressing the yield of Desmodium grown on the Mud Lane B, Pukalani and Kahua soils. This might suggest that sulfate has interfered with the utilization of molybdenum and phosphate by the plant, at least to some extent. There was a significant increase in Mo content of Desmodium grown on Mud Lane A soil due to the application of phosphate. This trend was also observed in the Mud Lane B and Kahua soils while no trend was noted in the Pukalani soil. The positive or negative effect of phosphate on Mo uptake thus seemed to depend on the initial level of phosphate in the soil. The initially high level of phosphate as found in Pukalani soil might have already enhanced the uptake of Mo to the maximum, and further addition thus failed to increase the uptake of this element. This enhancing effect of

phosphate was found to be more prominent when the soil is moderately acid, and no effect was found when the soil was neutral in reaction, as in the case of Pukalani soil. Phosphate, on the other hand, can also reduce the concentration of Mo in plants grown on a soil (Mud Lane A) which is low in available Mo through a dilution effect resulting from increased plant growth, but total Mo uptake is increased. This is considered an indirect effect.

Sulfate, in this experiment, greatly decreased Mo uptake through direct competition for absorption sites. Phosphorus uptake was decreased to a lesser extent. Sulfate depression of Mo uptake was exhibited by plants grown on the Mud Lane A, Mud Lane B, and Kahua soils. This depressing effect on Mo content was found to coincide with decreased plant yield. A Molybdenum-sulfate-phosphate interaction was also observed. When phosphate was not added, sulfate depressed Mo uptake to a considerable extent, and when phosphate was also added this depression effect was lessened. One possible explanation is that phosphate competed directly with sulfate for the adsorption sites on the plant roots, and hence reduced sulfate depression on Mo uptake.

The application of 2 lb/A of molybdenum increased the Mo content of Desmodium grown on Mud Lane A, Mud Lane B, and Pukalani soils to a level far beyond that which is considered toxic to livestock. This increase in Mo content of the plants grown on all four soils under study was highly significant. However, Mo addition to Kahua soil did not increase the content of this element in Desmodium to more than 5 ppm. There is no immediate explanation at the

present time as to why this increase was very small compared to that obtained in the three other soils. This soil has a moderate capacity for Mo fixation, but its capacity is considerably lower than that of Mud Lane A and Mud Lane B soils.

The last phase of this thesis is concerned with an investigation of Mo availability in the soil solution as affected by pH, phosphate and sulfate concentrations. Three soils were investigated. It was found that pH of the soils was the most dominant factor in regulating the availability of this element in the soil solution. Akaka soil exhibited the lowest availability of Mo because of its high adsorption capacity for Mo due to the presence of a large fraction of "active" amorphous iron and aluminum oxides. Naiwa soil adsorbed less Mo from solution than Akaka soil, and Catano sand adsorbed the least. The adsorption curves obtained with all three soils showed a very characteristic sigmoid shape. These findings are in close agreement with those of Jones (1957) who obtained a similar relationship between the adsorption of Mo from solution by soil colloids (as iron oxide, aluminum oxide, clay minerals) and the pH of the systems. Full recovery of Mo in solution was only obtained with Catano sand at pH 6.0. A plot of available molybdenum in a given soil against increasing pH results in a sigmoid curve exhibiting a sharp increase in availability at a specific pH value. This relationship between availability and soil pH is similar to that found between Mo content of Kikuyu grass and soil reaction as shown in Phase II

of this thesis. This finding fully supports the fact that the content of Mo in the plant depends solely on the "available Mo" that is present in the soil solution, and that Mo availability is further dependent on the soil reaction.

It was evident from the study employing a simplified soil system that phosphate is directly and significantly involved in anion exchange with molybdate as the amount of Mo in the solution was increased greatly when the phosphate concentration was increased. This finding was observed in the Akaka and Naiwa soils. Failure to obtain this effect in Catano sand was due to the fact that the pH of the soil system was adjusted too high to permit any appreciable amount of Mo to be adsorbed by the soil and exchange with phosphate. The molybdenum adsorption curves obtained were exponential in character.

Sulfate, although active in reducing the uptake of Mo by Desmodium in Phase III, was found to increase the availability of Mo to some extent in Naiwa soil, to a lesser degree in Akaka soil and had no effect in Catano sand. The curves obtained in plotting Mo adsorption against sulfate concentration were also exponential. It is evident that sulfate exchanges with Mo in the same way as phosphate; but the soil colloids appear to have less affinity for sulfate than for phosphate. Hence, phosphate is more actively exchanged and adsorbed than sulfate resulting in the difference in capacity of the two anions to release Mo to the soil solution. This effect of sulfate on molybdenum availability is certainly very small, and, in the presence of plants, will be

completely masked by its competition with Mo for adsorption sites as explained by Stout, et. al. (1951) and Barshad (1951). In general, sulfate depresses molybdenum uptake by plants.

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APPENDIX I

Mo content in ppm. of pasture species from 5 locations at various sampling dates.

	Locations				
Sampling Date	Ohaikea Vee	Pawaena	Pahala	Haau	Shipman
October, 1959	1.54	0.71	0.69	0.18	0.11
February, 1960	1.52	0.61	1.11	0.13	0.24
	1.31	0.61	0.67	0.19	0.21
	0.80	0.55	0.60	0.21	0.22
	1.55	0.73	0.40	0.24	0.08
	1.90	0.83	0.73	0.15	0.15
	1.87	0.95	0.73	0.19	0.03
	1.58	0.65	0.51	0.25	0.15
	2.57	1.18	0.42	0.17	0.06
	3.61	1.31	0.42	0.19	0.14
	1.79	1.19	0.88	0.20	0.15
	1.17	0.62	0.56	0.12	0.20
	1.78	0.97	0.44	0.22	0.29
	4.31	0.94	1.29	0.19	0.25
September, 1963	1.41	0.48	1.26	0.15	0.40
Average	1.91	0.82	0.71	0.19	0.18

APPENDIX II

Relation between molybdenum availability and pH of the soil system in three soils studied.

AKAKA (moist)		NAIWA		CATANO SAND	
pH	Mo in Soil ug./10 ml.	pH	Mo in Soil ug./10 ml.	pH	Mo in Soil ug./10 ml.
2.25	0.25	2.11	0.13	1.43	2.00
2.96	0.00	2.72	0.00	2.01	2.00
3.95	0.12	3.50	0.00	2.56	2.35
4.58	0.03	4.23	0.00	3.43	2.35
5.50	0.06	4.40	0.20	3.68	3.08
6.30	0.20	4.72	0.20	4.10	3.60
6.85	0.45	4.97	6.25	4.20	5.14
7.01	0.85	5.20	0.55	4.50	6.51
7.26	1.52	5.42	0.75	4.76	10.41
7.60	4.42	5.76	1.68	5.40	17.32
8.31	8.76	6.28	5.04	5.98	19.50
9.40	9.65	6.75	6.95	7.75	20.00
-	-	7.14	9.70	9.02	20.09
-	-	8.80	15.90	-	-

APPENDIX III

Relation between phosphate concentration and molybdenum availability in three soils studied.

P conc. ppm.	Mo remaining in solution (ug./10 ml.). Akaka (moist)	Naiwa	Catano Sand
0	0.19	1.54	19.30
100	1.73	2.60	19.50
200	3.01	4.21	19.28
300	3.52	5.19	19.40
400	4.48	6.30	19.70
500	4.55	6.57	19.50
1000	6.08	11.51	19.38
1500	7.01	12.18	19.59
2000	6.70	13.27	19.80
2500	6.53	13.20	19.60
3000	6.91	13.48	19.80

APPENDIX IV

Relation between sulfate concentration and molybdenum availability in three soils studied.

SO_4 conc. (ppm.)	Mo remaining in solution (ug./10 ml.). Akaka (moist)	Naiwa	Catano Sand
0	0.19	1.54	19.3
100	0.90	2.22	19.75
200	1.10	2.70	19.37
500	1.43	4.20	19.30
1000	1.20	4.38	19.03
1500	1.10	4.65	19.52
2000	1.40	4.52	19.45
2500	1.01	5.09	19.00
3000	1.18	4.50	19.20

ABSTRACT

Mo content in pasture species was found to be dependent on climatic and soil factors. Seasonal variation due to rainfall exerted an important effect on the fluctuating level of this element in pasture species. The highest content of Mo in samples collected at three-month intervals over a period of four years was found to occur when rainfall was highest, usually in the spring of each year, or around March. In a survey investigation concerned with the content of this element in pasture species it was found that the highest level of Mo, 39.0 ppm., occurred in orchard grass, this being the highest level thus far reported in the islands. There was a strong indication that Mo toxicity might have developed on certain areas of Kukaiau Ranch and Parker Ranch, both located on the northern side of the Island of Hawaii. The copper content of the pastures on some areas of these ranches was low and might also contribute to a very unfavorable quality of forage for animals. The high level of Mo in the forage seemed to be associated with high elevation and also low annual rainfall. It was evident that the degree of tropical weathering of soils occurring at higher elevations on the Island of Hawaii has not progressed to full maturity. Mo fixation by these soils was expected to be low due to the low degree of weathering, which is usually associated with a low amount of sesquioxides. This condition might result in high available Mo in the soil solution, thus permitting plants growing at this elevation to accumulate Mo

in greater quantity than at the lower elevations. The Mo content of kikuyu grass was found to correlate well with water extractable Mo in the soils, and also with soil pH. However, total molybdenum in the soil did not evidence a similar relationship. Also, there was no relationship between total copper in the soils and copper content of the plants.

Molybdenum application was found highly effective in increasing the Mo content of Desmodium tortuosum grown on four soils in the greenhouse. Phosphate application also enhanced the uptake of Mo by the plant. This enhancing effect was very marked in soils where the pH was moderately acid, the effect not being found in a soil with neutral reaction. Sulfate application gave a reverse effect to that of phosphate in that it decreased the uptake of Mo by the plant, sometimes as much as two-fold. This depressing effect of sulfate on Mo adsorption was found in soils with a moderately acid reaction and not found in a soil with neutral reaction which was associated with a high initial level of Mo and P. The effect of P on increasing the Mo content of plants suggested an anion exchange process between P and Mo, thus rendering Mo more available than the sorbed forms. This lead to an experiment with a simplified soil system to measure the availability of Mo in soil solution as a function of P concentration. From the results obtained it was evident that P significantly increased the amount of Mo in the solution phase, probably by an anion exchange process. Sulfate concentration had a similar effect, but to a lesser extent. The influence of pH on the adsorption of Mo by soils was also determined, and the

results obtained were similar to those of Jones (1957). Comparing the effects among pH (OH ion concentration), sulfate and phosphate, soil pH was found to be the most important single factor controlling the level of Mo availability in the soil solution.